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Adipose tissue and skeletal muscle as endocrine organs: Role of cytokines in health and disease

Edited by
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Published in
Frontiers in Physiology

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ISSN 1664-8714
ISBN 978-2-83250-987-6
DOI 10.3389/978-2-83250-987-6

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Adipose tissue and skeletal muscle as endocrine organs: Role of cytokines in health and disease

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Citation

De Oliveira Duarte, A. C. G., Lira, F., Speretta, G. F. F., Teixeira, A. M., eds. (2022). *Adipose tissue and skeletal muscle as endocrine organs: Role of cytokines in health and disease*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83250-987-6

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frontiers | Frontiers in Physiology

TYPE Editorial
PUBLISHED 23 November 2022
DOI 10.3389/fphys.2022.1069431

Check for updates

OPEN ACCESS

EDITED AND REVIEWED BY
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SPECIALTY SECTION
This article was submitted to Metabolic Physiology, a section of the journal Frontiers in Physiology

RECEIVED 13 October 2022
ACCEPTED 19 October 2022
PUBLISHED 23 November 2022

CITATION
Duarte ACGDO, Speretta GF, Teixeira AM and Lira FS (2022), Editorial: Adipose tissue and skeletal muscle as endocrine organs: Role of cytokines in health and disease.
Front. Physiol. 13:1069431.
doi: 10.3389/fphys.2022.1069431

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Editorial: Adipose tissue and skeletal muscle as endocrine organs: Role of cytokines in health and disease

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KEYWORDS

adipose tissue, skeletal muscle, adipokines, exercise trainig, health, diseases

Editorial on the Research Topic
Adipose tissue and skeletal muscle as endocrine organs: Role of cytokines in health and disease

Introduction

One of the central factors to maintaining energy regulation is preserving the healthy functioning of white adipose tissue. An excess of this tissue is associated with metabolic dysfunctions, which promote a positive energy balance and elevated inflammatory adipokines, causing obesity and metabolic syndrome. Such disturbances in energy homeostasis can induce specific immune responses, which can influence the pathophysiology of several diseases. Thus, cytokines that promote physiological responses in the body have been examined as potential therapeutic agents for energy regulation. A significant number of these cytokines are secreted by non-adipose but metabolically active tissues, such as skeletal muscle (myokines) and the liver and may act in a corrective manner on inflammatory adipokines involved in the development of obesity. Thus, several cytokines can inhibit or suppress the actions of adipokines associated with the inflammatory obesity phenotype, as occurs with some myokines.

In this regard, the importance of physical activity cannot be understated since the benefits provided go far beyond mechanical adaptations and control of skeletal muscle energy homeostasis. The existence of humoral components such as paracrine, autocrine, and endocrine regulators that

control skeletal muscle adaptive processes is also of benefit. Among the endocrine functions that are attributed to myokines, the regulation of body weight favoring a negative energy balance, the reduction of chronic low-grade inflammation, and the regulation of insulin signaling are promising for the treatment of chronic diseases such as obesity and diabetes mellitus. Recently, the number of myokines that are secreted in response to muscle contraction has been growing steadily, and new factors have been identified.

This Frontiers Research Topic includes a broad range of articles in the following areas: 1) metabolic regulations that can occur between the adipose tissue and the muscle and that are influenced by exercise; 2) the relationships between various myokines and adipokines in response to exercise; 3) metabolic disorders related to adipose tissue; and 4) mechanisms involved in the energy balance regulation process.

Therefore, knowledge of factors related to the metabolism of physical exercise, which regulates energy in the adipose tissue through interactions between metabolically active tissues, allows consolidation of this topic and may contribute to the recovery of energy homeostasis and mechanisms which, until now, have not been completely understood.

In this Research Topic, four review papers were published. Lyu et al. discussed the functions and mechanisms related to how low-level laser therapy, used for tendon repair, activates a large number of VEGF and promotes angiogenesis under hypoxia, increasing the amount of collagen type III by promoting the proliferation of fibroblasts. Throughout the remodeling phase, LLLT primarily activates M2 macrophages and downregulates inflammatory factors, thus reducing inflammatory responses. de França et al. examined the potential role of regular physical exercise as a treatment during the development of vitiligo, highlighting certain clinically relevant markers that can be analyzed in a new research avenue. de Jesus Alves et al. highlighted changes in cytokine concentrations following long-distance running and their close relationship with the running volume. The cytokines modulate compounds that play a fundamental role in the maintenance of homeostasis and cell signaling. Cai et al. discussed the ectodysplasin A/ectodysplasin A receptor system function and the physiological and pathological roles of its receptors in multiple diseases.

Two original papers discussed the effect of exhaustive exercise on immune responses. de Sousa et al. examined the course of time and the role of exercise-induced cytokines in muscle damage and repair after a marathon race. This study demonstrated that classical anti-inflammatory mediators (IL-10, IL-8, and IL-6) induced by exercise are associated with myokine response both immediately after the race and in the recovery period and may affect muscle tissue repair dynamics. Lobo et al. demonstrated that a single bout of fatiguing aerobic exercise induced similarly pronounced immunological responses in both women and men.

Three original papers demonstrated the effect of regular exercise on aging or obesity. Peres et al. explored the potential anticarcinogenic effect of plasma (*in vitro*) in older adults after exercise. The authors observed that adaptations in the blood factors of institutionalized older adults might alter cell viability and proliferation by targeting mitochondrial ROS in the prostate cancer cell line. Farinha et al. observed that both interval aerobic exercise and combined exercise programs appeared to be more effective than a continuous aerobic exercise program in decreasing chronic low-grade inflammation by mediating the production of higher levels of anti-inflammatory cytokines. However, the authors highlighted that the differences observed between the exercising groups were small and may not be clinically significant. Regarding obesity, Bonfante et al. examined the effects of the acute/chronic responses of combined training on serum pro-thermogenic/anti-inflammatory inducers and their relationship with both the nourished and fasting state in overweight type 2 diabetic individuals.

Three original papers studied experimental animal models. He et al. observed exercise-enhanced cardiac function in mice, *via* the FNDC5/Irisin-dependent mitochondrial turnover pathway, with radiation-induced heart disease. Faria et al. showed that exercise-induced melatonin potentiates increased skeletal muscle PGC-1α and optimized glycogen replenishment. Finally, da Costa et al. demonstrated that dietary intervention and moderate-intensity continuous training led to changes in the inflammatory profile of visceral adipose tissue but not in the skeletal muscle in diet-induced obese rats.

This Research Topic highlights the essential roles of adipose tissue and skeletal muscle regarding cytokines released to the bloodstream and their metabolic consequences in subjects with chronic diseases or who are healthy. Regular physical exercise leads to reduced metabolic and inflammatory disruptions and can be adopted as a nonpharmacologic treatment. Scientists are thus collaborating to find new directions for promoting a better quality of life as well as a physically active life to treat chronic disease, reinforcing that regular physical exercise can alleviate the complex conditions reported in this Research Topic.

Author contributions

AD, GF, AT and FL participated all process. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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frontiers in Physiology

ORIGINAL RESEARCH
published: 15 October 2021
doi: 10.3389/fphys.2021.752144

Time Course and Role of Exercise-Induced Cytokines in Muscle Damage and Repair After a Marathon Race

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Endurance exercise induces an increase in the expression of exercise-induced peptides that participate in the repair and regeneration of skeletal muscles. The present study aimed to evaluate the time course and role of exercise-induced cytokines in muscle damage and repair after a marathon race. Fifty-seven Brazilian male amateur marathon finishers, aged 30–55 years, participated in this study. The blood samples were collected 24 h before, immediately after, and 24 and 72 h after the São Paulo International Marathon. The leukogram and muscle damage markers were analyzed using routine automated methodology in the clinical laboratory. The plasma levels of the exercise-induced cytokines were determined using the Human Magnetic Bead Panel or enzyme-linked immunosorbent assays [decorin and growth differentiation factor 15 (GDF-15)]. A muscle damage was characterized by an increase in plasma myocellular proteins and immune changes (leukocytosis and neutrophilia). Running the marathon increased interleukin (IL)-6 (4-fold), IL-8 (1.5-fold), monocyte chemoattractant protein-1 (2.4-fold), tumor necrosis factor alpha (TNF-α) (1.5-fold), IL-10 (11-fold), decorin (1.9-fold), GDF-15 (1.8-fold), brain-derived neurotrophic factor (BDNF) (2.7-fold), follistatin (2-fold), and fibroblast growth factor (FGF-21) (3.4-fold) plasma levels. We also observed a reduction in musclin, myostatin, IL-15, and apelin levels immediately after the race (by 22–36%), 24 h (by 26–52%), and 72 h after the race (by 25–53%). The changes in BDNF levels were negatively correlated with the variations in troponin levels ($r = -0.36$). The variations in IL-6 concentrations were correlated with the changes in follistatin ($r = 0.33$) and FGF-21 ($r = 0.31$) levels after the race and with myostatin and irisin levels 72 h after the race. The changes in IL-8 and IL-10 levels had positive correlation with variation in musclin ($p < 0.05$). Regeneration of exercise-induced muscle damage involves the participation of classical inflammatory mediators, as well as GDF-15, BDNF, follistatin, decorin, and FGF-21, whose functions include myogenesis, mytophagia, satellite cell activation, and downregulation of protein degradation. The skeletal muscle damage markers were not

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *02 August 2021*
Accepted: *13 September 2021*
Published: *15 October 2021*

Citation:
de Sousa CAZ, Sierra APR, Martínez Galán BS, Maciel JFdS, Manoel R, Barbeiro HV, Souza HPd and Cury-Boaventura MF (2021) Time Course and Role of Exercise-Induced Cytokines in Muscle Damage and Repair After a Marathon Race. Front. Physiol. 12:752144. doi: 10.3389/fphys.2021.752144

associated to myokines response. However, BDNF had a negative correlation with a myocardial damage marker. The classical anti-inflammatory mediators (IL-10, IL-8, and IL-6) induced by exercise are associated to myokines response immediately after the race and in the recovery period and may affect the dynamics of muscle tissue repair.

Keywords: muscle damage, myocardial damage, inflammation, endurance exercise, myokines, muscle repair

INTRODUCTION

The mechanical and metabolic stress (mitochondrial dysfunction and challenge by energy ADP/ATP ratio), induced by the repetitive contractions of muscle fibers, causes a disruption of the sarcolemma and extracellular matrix, swelling of mitochondria, dilation of the transverse tubule system, and fragmentation of the sarcoplasmic reticulum, thereby promoting the increased permeability of the membrane and efflux of myocellular proteins into the blood circulation (Peake et al., 2017; Hody et al., 2019). The loss of calcium homeostasis, oxidative stress, increased calpain activity, and inflammation contribute to muscle injury (Hody et al., 2019). An increase in the levels of systemic inflammatory mediators, such as interleukin (IL)-1 beta, IL-6, IL-8, IL-1ra, and IL-10, is induced during the muscle damage due to endurance exercise. However, the cellular sources of these mediators, such as endothelial cells, pericytes, fibroblasts, neutrophils and monocytes/macrophages, or skeletal muscle cells, utilize the inflammatory mediators in the process of muscle repair and regeneration (Peake et al., 2015).

In addition to the classical inflammatory mediators, the changes have been observed in more than 650 myokines in response to muscle contraction and differentiation, with autocrine, paracrine, and endocrine actions (Whitham and Febbraio, 2016; Safdar and Tarnopolsky, 2018; Piccirillo, 2019; Bay and Pedersen, 2020; Laurens et al., 2020). Despite the identification of hundreds of myokines, biological function has only been described in a small portion of myokines. Many of these myokines have paracrine biological functions in muscle protein synthesis or degradation, proliferation and differentiation of myoblasts, activation of satellite cells, organization and remodeling of the extracellular matrix, as well as modulation of muscle wasting, repair, and regeneration (Hoffmann and Weigert, 2017; Lee and Jun, 2019; Laurens et al., 2020). The cellular sources of myokines include myocytes, satellite cells, endothelial cells, residential macrophages, and fibroblasts (Hoffman and Weigert, 2017). The myokines primarily studied after aerobic exercise include myostatin, IL-6, irisin, IL-15, growth differentiation factor 15 (GDF-15), brain-derived neurotrophic factor (BDNF), fibroblast growth factor (FGF)-21, apelin, angiopoietin-like protein 4, and decorin (Safdar and Tarnopolsky, 2018; Piccirillo, 2019; Bay and Pedersen, 2020; Laurens et al., 2020). The levels of systemic hepatokines, such as follistatin, FGF-21, and angiopoietin-like protein 4, have also been studied during and after the acute exercise (Gonzalez-Gil and Elizondo-Montemayor, 2020). The release of exercise-induced peptides is dependent on the type of exercise and training protocol (Piccirillo, 2019; Domin et al., 2021).

Understanding the time course of a variety of different exercise-induced peptides can help highlight the circulatory markers that participate in the different phases of the muscle damage and repair, such as pro and anti-inflammatory phase, muscle and connective tissue remodeling as well as synthesis and protein degradation after long-distance exercise. The present study is the first study to determine and correlate a large number of muscle damage markers and inflammatory and tissue repair mediators in long-distance runners. Our hypothesis is that the extent of muscle damage may influence the inflammatory response, which in turn may affects the dynamics of muscle tissue repair during the recovery period.

The present study aimed to evaluate the time course and relationship of exercise-induced cytokines and muscle damage markers after a marathon race. An understanding of the course of the release of exercise-induced peptides on circulation and their association during muscle damage and repair may contribute to elucidate the endurance exercise muscle adaptations and may yield potential molecular therapeutic targets to treat the myopathies that involve mitochondrial dysfunctions.

MATERIALS AND METHODS

Subjects

Fifty-seven amateur Brazilian male marathon finishers, aged 30–55 years, participated in this study. The recruitment of the volunteers was performed by the São Paulo International Marathon Organization (2017, Yescom, BRA) by mailing and the volunteers filled a form containing personal data, such as age, e-mail, address, and phone number. The researchers contacted the volunteers by phone to inform the more details of the collection steps, such as four blood collection and a cardiopulmonary exercise test to confirm interest and availability of a runner to participate in the research. Then, the runners were randomized after training and medical history. The exclusion criteria included the use of medication for cardiac, metabolic, pulmonary, or kidney injury, use of alcohol or any kind of drugs and pathologies, such as systemic arterial hypertension, liver, kidney, metabolic, inflammatory, or neoplastic diseases. The inclusion criteria were having already participated in one or more marathon or half marathon and being training for more than 30 km per week. The subjects were informed of the experimental procedures and possible risks and signed a term of informed consent approved by the Ethics Committee of Dante Pazzanese Institute of Cardiology, Brazil (Permit Number: 979/2010), in accordance

with the Declaration of Helsinki. A cardiopulmonary exercise test (CPET) was performed 3–21 days before the marathon race using a treadmill protocol (TEB Apex 200, TEB, São Paulo, Brazil, speed 0–24 km/h, grade 0–35%). The test was performed in 1% fixed slope and speed began with 8 km/h increasing 1 km/h per minute until the maximal exhaustion of the runner. An expired gas analysis was performed in a breath-by-breath system (Quark CPET, Cosmed, Rome, Italy). During the test, the runners were monitored with a standard 12-lead electrocardiogram (4 limb and 6 thorax electrodes, ECG, TEB, São Paulo, Brazil) to check for the possible cardiac changes during exertion. All the runners recruited completed the International Marathon of São Paulo 2017.

The measurements of total body mass (kg), height (cm), and Body Mass Index (BMI, kg/m^2) were conducted 1-day before the marathon race at Cruzeiro do Sul University, São Paulo, Brazil, according to the International Society for the Advancement of Kinanthropometry and expressed as the mean ± SEM. The body composition (percentage of fat mass and free fat mass) was assessed by the bioimpedance analysis (Biodynamics Corporation, Seattle, WA, USA, 310e) with the runners fasting for at least 6 h 1-day before the marathon.

The blood samples (30 ml) were collected in vacuum tubes containing an anticoagulant [0.004% ethylenediaminetetraacetic acid [EDTA]]. Before, 24 h after, and 72 h after blood samples were collected at the Institute of Physical Activity and Sports Science (Cruzeiro do Sul University) and 20 ml forwarded to the clinical laboratory (Associação Fundo de Incentivo à Pesquisa, AFIP) for immediate skeletal and cardiac muscle damage markers and leukogram analyses and 10 ml were immediately centrifugated for plasma collection, and storage at −80°C, for the subsequent exercise-induced cytokine analyses. The blood samples immediately after the race were collected from a research area located close to the finish line and 20 ml forwarded to the clinical laboratory (Associação Fundo de Incentivo à Pesquisa, AFIP) and 10 ml to the Institute of Physical Activity and Sports Science for the same analyses.

Marathon Race

The São Paulo International Marathon (2017) started at 07:30 a.m. on April 9. Fluid ingestion was allowed *ad libitum* during the race. Water was available every 2–3 km on the running course; sports drinks were available at 12, 21.7, 33, and 42 km; and a potato was available at 28.8 km. The weather parameters between 07:00 a.m. and 02:00 p.m. were as follows: average temperature, 19.8°C; average relative humidity, 72.8% (the National Institute of Meteorology, Ministry of Agriculture, Livestock, and Supply, Brazil).

Muscle Damage Markers and Leukogram

The muscle damage markers were evaluated using a routine automated methodology in the Clinical Laboratory (Associação Fundo de Incentivo à Pesquisa, AFIP), immediately after the blood collection (20 ml). The creatine kinase (CK) and lactate dehydrogenase (LDH) activities were determined *via* a kinetic assay; troponin (I), N-terminal pro B-type natriuretic peptide (NT-proBNP), and myoglobin levels, and creatine kinase MB (CKMB) activity, were evaluated using a chemiluminescence assay. Leukogram was constructed *via* measurement using the cytochemical/isovolumetric method, and the C-reactive protein (CRP) levels were quantified *via* immunoturbidimetry assay.

Determination of Cytokines Induced by Exercise

The plasma levels of interleukin (IL)-1ra, IL-4, IL-10, tumor necrosis factor alpha (TNF-α), vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), interferon-gamma (IFN-gamma), macrophage inflammatory protein-1 (MIP-1), and monocyte chemoattractant protein-1 (MCP-1) were determined using the MILLIPLEX® Human Cytokine/Chemokine Magnetic Bead Panel (HCYTOMAG-60K, EMD Millipore Corporation, MA, USA). The plasma levels of apelin, irisin, BDNF, myostatin, musclin, follistatin, IL-6, IL-15, and FGF-21 were determined using the MILLIPLEX® Human Myokine Magnetic Bead Panel (HCYTOMAG-56K, EMD Millipore Corporation, MA, USA), according to the instructions of the manufacturer. The decorin and growth differentiation factor 15 (GDF-15) levels were determined ***via*** enzyme-linked immunosorbent assays (Duoset-ELISA, R&D Systems, USA).

The intra assay precision (mean of the percentage of coefficient variation) described by the protocols of the manufacturer were <3% to IL-1ra, IL-6, IL-10, IL-15, FGF-2, IFN-gamma, MIP-1 and were <10% to TNF-α, VEGF, MCP-1, apelin, irisin, BDNF, myostatin, musclin, follistatin, FGF-21, GDF-15, and decorin.

Statistical Analyses

The statistical analyses were performed using GraphPad Prism (GraphPad Prism version 9, San Diego, CA, USA). The normality of the data distribution was determined using the Kolmogorov–Smirnov test and the normality was rejected. The general and training characteristics were described as the mean ± SEM. The statistical analyses were evaluated using one-way repeated measures ANOVA test, Geisser–Greenhouse correction for sphericity and Holm–Sídák post-test for multiple comparisons between before vs. immediately after, 24 and 72 h after the race. The non-parametric Spearman correlations were determined between the changes in muscle damage marker levels and general characteristic and training characteristic data; between the leukogram and general characteristic and training characteristic data; between the cytokine levels and general characteristic and training characteristic data (before, immediately after the race, or 24 h after the race); and between the changes in cytokine and muscle damage marker levels. The changes (after the race–before the race) in the levels of troponin, proBNP, LDH, leukogram, IL-6, IL-8, MCP-1, TNF-alpha, IL-10, apelin, decorin, GDF-15, BDNF, follistatin, and FGF-21 were calculated. The variations (24 h after the race–before the race) in CK and CKMB activities, and CRP level were calculated and the changes (72 h after the race–before the race) in IL-15, irisin, myostatin, apelin, and IL-6 levels were calculated. The statistical significance was assumed at $p < 0.05$. In the graph, the absolute values presented comprise the minimum, maximum, median,

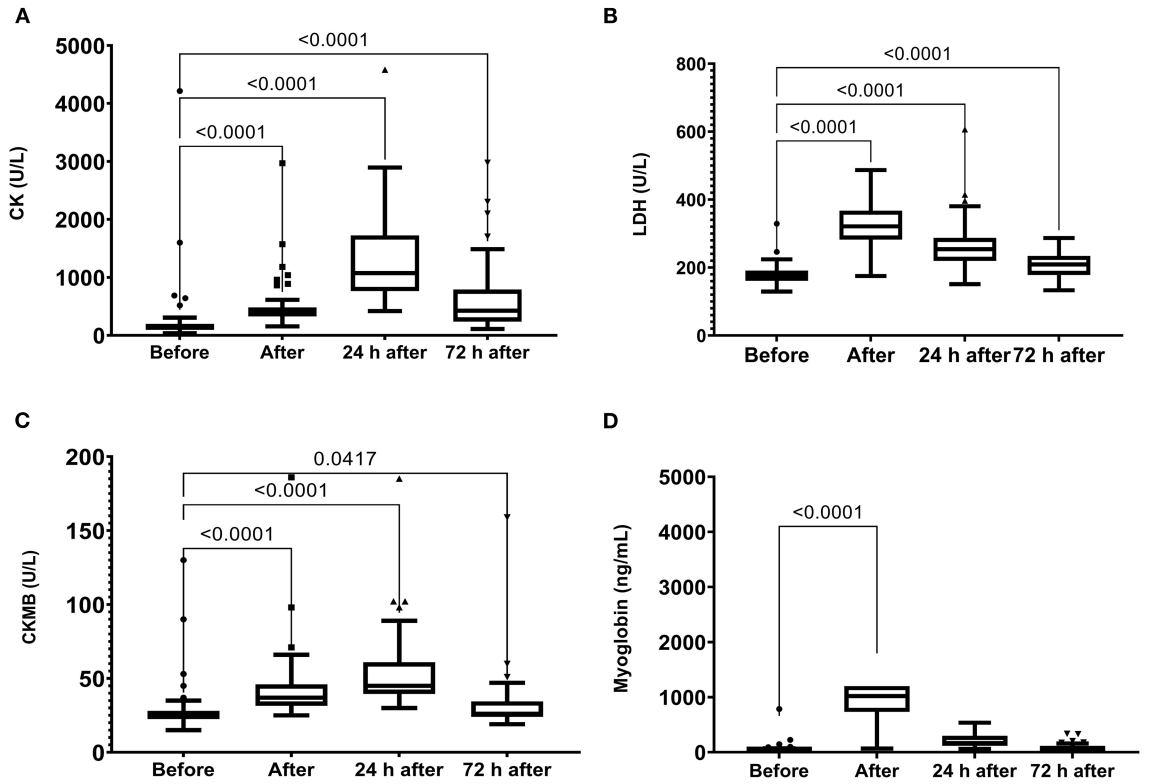

FIGURE 1 | Creatine kinase (CK) **(A)**, lactate dehydrogenase (LDH) **(B),** Creatine kinase MB (CKMB) **(C)** activities and myoglobin **(D)** plasma levels immediately after, 24 h after, and 72 h after the race. Creatine Kinase, CK, Lactate Dehydrogenase, LDH. The values are presented as minimum, maximum, median, and outliers of 53 runners (Box plot, Tukey).

and outliers, with respect to the 40–57 runners. To perform one-way repeated measures ANOVA test, four runners were excluded from the analysis of leukogram, CRP, and muscle damage markers for not having completed the four blood collections and 10–13 runners were excluded from the analysis of cytokines with no detectable value before, after, 24 h, or 72 h after the race.

RESULTS

General Characteristics

The general and training characteristics of amateur marathon runners are summarized as follows: age, 41.1 ± 0.9 years; weight, 74.8 ± 2.7 kg; height, 1.73 ± 0.01 m; BMI, 24.3 ± 0.5 kg/m^2; percentage of fat mass, 21.8 ± 0.6%; free fat mass, 57.7 ± 0.9 kg; race time 248.9 ± 5.8 min, training experience, 7.4 ± 0.7 years; time in 10 km race, 46.5 ± 0.82 min; frequency of training, 4.2 ± 0.15 times/week; and training volume, 55.1 ± 4.9 km/week. The CPET parameters are summarized as follows: maximum speed of runners was 18.9 ± 0.3 km/h; time of exhaustion 11.5 ± 0.3 min; anaerobic threshold oxygen consumption (VO_2 AT), 34.5 ± 1.0 ml/kg/min; respiratory compensation point oxygen consumption (VO_2 RCP), 53.9 ± 1.2 ml/kg/min; and peak oxygen consumption (VO_2 peak), 56.0 ± 1.3 ml/kg/min.

Muscle Damage Markers and Leukogram

The skeletal muscle damage was characterized by an increase in CK, LDH, and CKMB activity and myoglobin concentration after the race until 72 h after the race (**Figure 1**). In addition, we observed myocardial damage by the elevation of troponin and proBNP levels after the race (**Figure 2**). The total leukocytes, neutrophils, and monocytes were elevated after the race and 24 h after the race (**Figures 3A–C**); level of lymphocytes decreased after the race (**Figure 3D**), demonstrating immune changes after the race. We also observed an increase in the CRP levels, 24 h after the race, until 72 h after the race (from 0.83 ± 0.31 to 2.2 ± 0.2 to 0.9 ± 0.04 mg/dl).

The variations in the total number of leukocytes, neutrophils, and monocytes were correlated with the variations in CKMB and LDH activities and troponin and myoglobin levels (**Table 1**). The changes in CRP were correlated with the variations in troponin ($p = 0.043$, $r = -0.27$) and NT-proBNP levels ($p = 0.037$, $r = -0.38$), indicating a suitable marker of myocardial damage after the race.

Time Course of Exercise-Induced Cytokines

Running the marathon led to the elevations in IL-6, IL-8, MCP-1, IL-10, and TNF-α plasma levels (**Figure 4**). IL-6 concentration

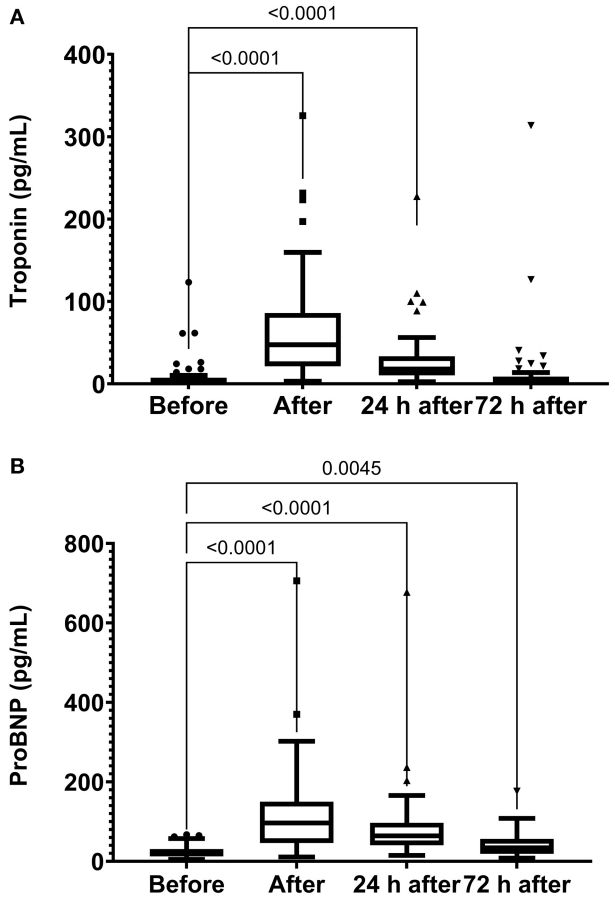

FIGURE 2 | Troponin **(A)** and NT-proBNP **(B)** levels immediately after, 24 h after, and 72 h after the race. N-Terminal pro B-type natriuretic peptide, proBNP. The values are presented as minimum, maximum, median, and outliers of 53 runners (Box plot, Tukey).

reduced 24 and 72 h after the race (**Figure 4A**) and IL-8, MCP-1, TNF-α, and IL-10 plasma levels returned to the basal levels 24 h after the race (**Figures 4B–E**). The decorin, GDF-15, BDNF, follistatin, and FGF-21 levels were increased immediately after the race (**Figure 5**). Decorin elevated 72 h after the race (**Figure 5A**) and follistatin decreased 24 and 72 h after the race (**Figure 5D**). The GDF-15, BDNF, and FGF-21 levels returned to the basal levels 24 h after the race (**Figures 5B,C,E**).

In addition, we observed a reduction in musclin, myostatin, apelin, and IL-15 levels immediately after the race, which were maintained until 72 h after the race (**Figure 6**).

We did not observe the changes in plasma levels of VEGF, FGF-2, IFN- gamma, IL-4, IL-1ra, irisin, and MIP-1-alpha (data not shown).

Correlation: General Characteristics

The changes in the CRP levels were correlated with body mass ($p = 0.012$, $r = -0.33$), BMI ($p = 0.018$, $r = -0.31$), and the percentage of fat mass ($p = 0.0007$, $r = -0.43$), and VO_{2peak} (0.005, $r = -0.37$). We observed no association between the changes in muscle damage markers and body composition (data not shown).

The changes in the BDNF levels were positively correlated with body mass, BMI, percentage of fat mass, and free fat mass, while the variations in IL-10 levels exhibited a negative correlation with these body composition parameters (**Figure 7**), suggesting a greater anti-inflammatory response and impairment of the BDNF response in runners with lower free fat mass. In addition, the changes in myostatin exhibited a negative correlation with BMI ($p = 0.24$, $r = -0.32$).

The training experience was positively correlated with the changes in the total leukocyte and neutrophil counts and LDH activity ($p < 0.05$, $r = 0.30$). VO_{2peak} correlated with the variations in monocytes ($p = 0.018$, $r = -0.31$). The training experience was negatively correlated with the changes in those BDNF ($p = 0.008$, $r = -0.38$) and GDF-15 levels ($p = 0.02$, $r = -0.31$) and positively correlated with the IL-10 levels ($p = 0.025$, $r = 0.35$). The training experience exhibited a positive correlation with the absolute values of GDF-15, musclin, myostatin, and irisin levels before the race ($p < 0.05$, $r = 0.30$). The race time was negatively correlated with the IL-10, myostatin, and irisin levels ($p < 0.05$, $r = -0.33$).

Correlations Between Exercise-Induced Cytokines Levels and Muscle Damage Markers

After the race, the changes in LDH activity correlated with the variations in those IL-10 ($p = 0.010$, $r = 0.49$) and TNF-α ($p = 0.03$, $r = 0.33$). The changes in CKMB activity also correlated with the variations in IL-10 levels ($p = 0.010$, $r = 0.39$). No association was found between the skeletal muscle damage markers and myokines analyzed in this study. Moreover, we observed a negative correlation between the changes in troponin and BDNF levels ($p = 0.012$, $r = -0.37$), indicating lower myocardial damage marker levels in the runners with greater BDNF response.

Correlations Associated With Exercise-Induced Cytokine Levels

The changes in IL-6 concentration were correlated with the changes in follistatin ($p = 0.009$, $r = 0.37$) and FGF-21 levels ($p = 0.007$, $r = 0.40$). The changes in IL-8 and IL-10 levels had positive correlated with the variation in musclin ($p = 0.013$, $r = 0.39$ and $p = 0.034$, $r = 0.34$, respectively). The changes in follistatin concentration were positively correlated with the changes in BDNF ($p < 0.0001$, $r = 0.55$) and FGF-21 ($p = 0.041$, $r = 0.31$). The variations in musclin had a strong correlation with the variations in Apelin ($p < 000.1$, $r = 0.70$).

The changes in FGF-21 levels were negatively correlated with the variations in those of decorin ($p = 0.039$, $r = -0.32$) and apelin levels ($p = 0.019$, $r = -0.35$).

In the recovery period, the changes in IL-6 (72 h after the race) were correlated with the changes in those myostatin, irisin, and apelin levels ($p < 0.0001$). The changes in IL-15 also were correlated with in those myostatin (0.038), irisin ($p < 0.0001$), and apelin levels ($p < 0.0001$). The changes in apelin and irisin

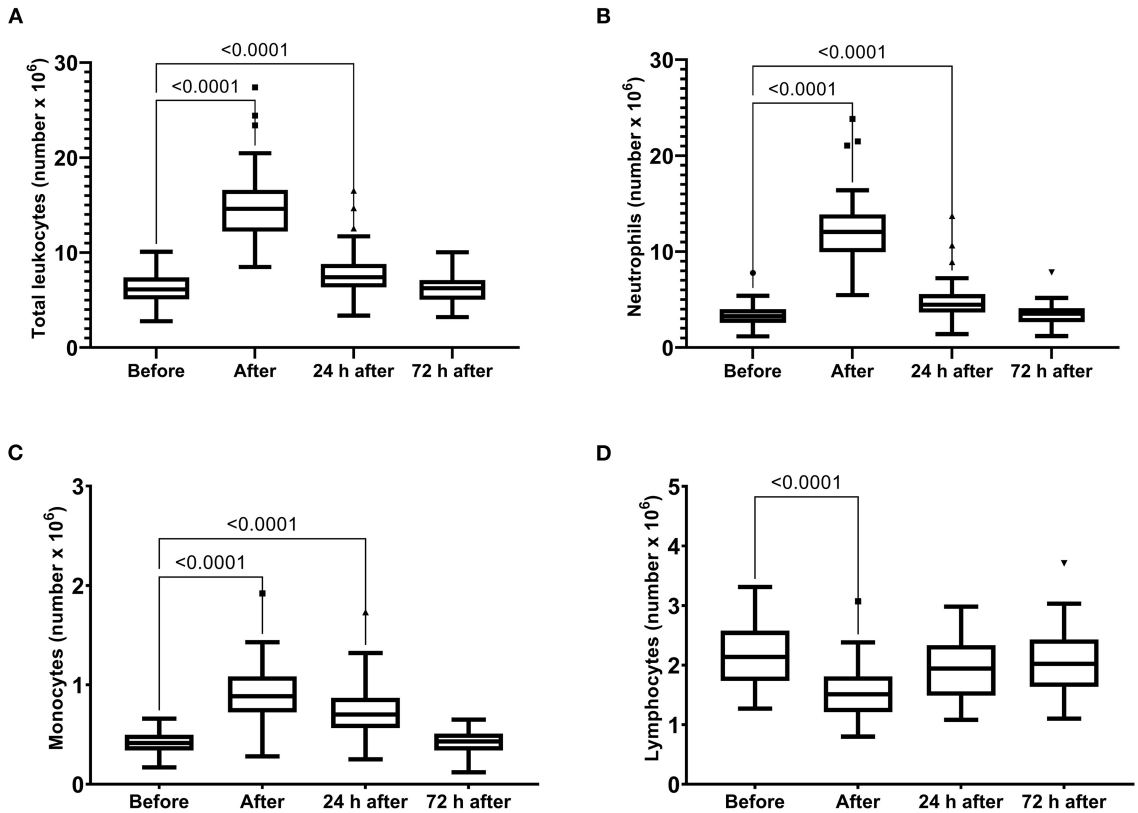

FIGURE 3 | Number of total leukocytes **(A)**, neutrophils **(B)**, monocytes **(C)** and lymphocytes **(D)** immediately after, 24 h after, and 72 h after the race. The values are presented as minimum, maximum, median, and outliers of 53 runners (Box plot, Tukey).

TABLE 1 | Correlation of changes in the number of leukocytes and changes in the muscle damage markers.

	Δ Number of leukocytes		Δ Number of neutrophils		Δ Number of monocytes	
	p	*r*	*p*	*r*	*p*	*r*
Δ CKMB	0.0006	0.44	0.0001	0.48	0.037	0.27
Δ Troponin	0.013	0.36	0.019	0.31	0.0014	0.41
Δ Myoglobin	0.032	0.28	0.0011	0.33	NS	NS
Δ LDH	0.0004	0.45	<0.0001	0.5	NS	NS

CKMB, Creatine kinase MB; LDH, lactate dehydrogenase, NS, not significantly; Δ, Delta of variation.

also had strong correlation with the changes in myostatin ($p < 0.0001$) (**Figure 8**).

DISCUSSION

Immediately after the race, we observed leukocytosis, neutrophilia, and an increase in the muscle damage markers, IL-6, IL-8, IL-10, TNF-α, MIP-1, decorin, GDF-15, BDNF, follistatin, and FGF-21, owing to a reduction in the myostatin, musclin, IL-15 and apelin levels which were maintained reduced 72 h after the race (**Figure 9**). The muscle damage marker, plasmatic LDH activity, correlated with the mediators of inflammation (IL-10 and TNF-α) but not with myokines response. The classical anti-inflammatory mediators induced by exercise (IL-6 and IL-10) and IL-8 seem to be associated with myokines that affect the muscle repair. IL-10 and IL-8 response was associated with the musclin response, while IL-6 response was positively correlated with the FGF-21 and follistatin response after the race and with myostatin, apelin, and irisin response in the recovery period. Moreover, BDNF had a negative correlation with the troponin levels and further studies should be carried out to verify the role of BDNF levels in myocardial damage-induced by the race.

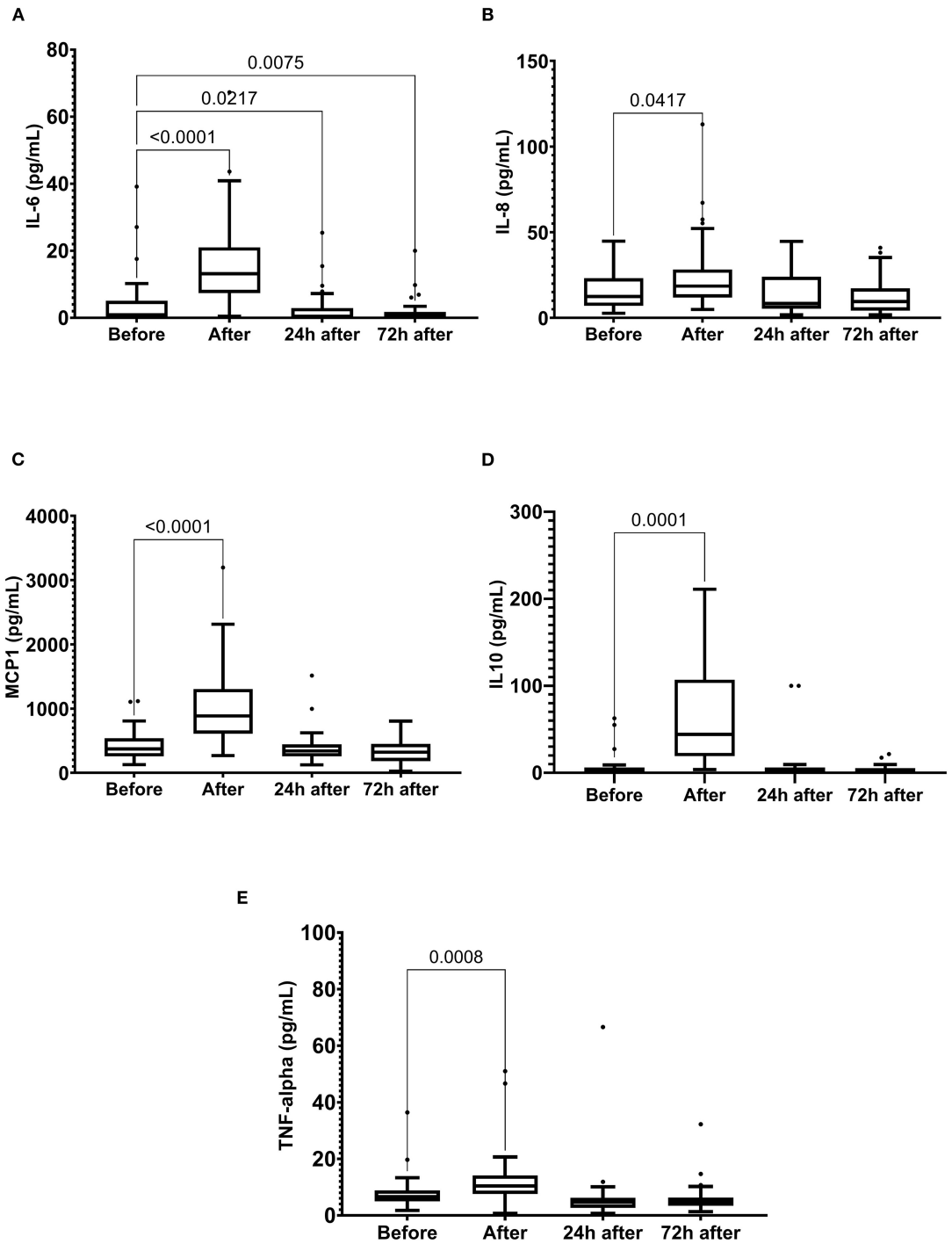

FIGURE 4 | The plasma levels of interleukin (IL)-6 **(A)**, IL-8 **(B)**, monocyte chemoattractant protein-1 (MCP1) **(C)**, IL-10 **(D)**, and tumor necrosis factor alpha (TNF-α) **(E)** immediately after, 24 h after, and 72 h after the race. The values are presented as minimum, maximum, median, and outliers of 40 runners (IL-6) or 43 runners (MCP1, TNF-alpha, and IL-10) (Box plot, Tukey).

Leukocytosis after long-distance exercise is a result of hemodynamic and catecholamine induced demargination of vascular and pulmonary pools followed by a cortisol-induced release of neutrophils from the bone marrow. In the recovery period, these stress hormones also contribute to lymphocytopenia and both innate and acquire immune cell dysfunction (Nieman and Mitmesser, 2017; Jones and Davison, 2019; Suzuki et al., 2020). Muscle tissue infiltration

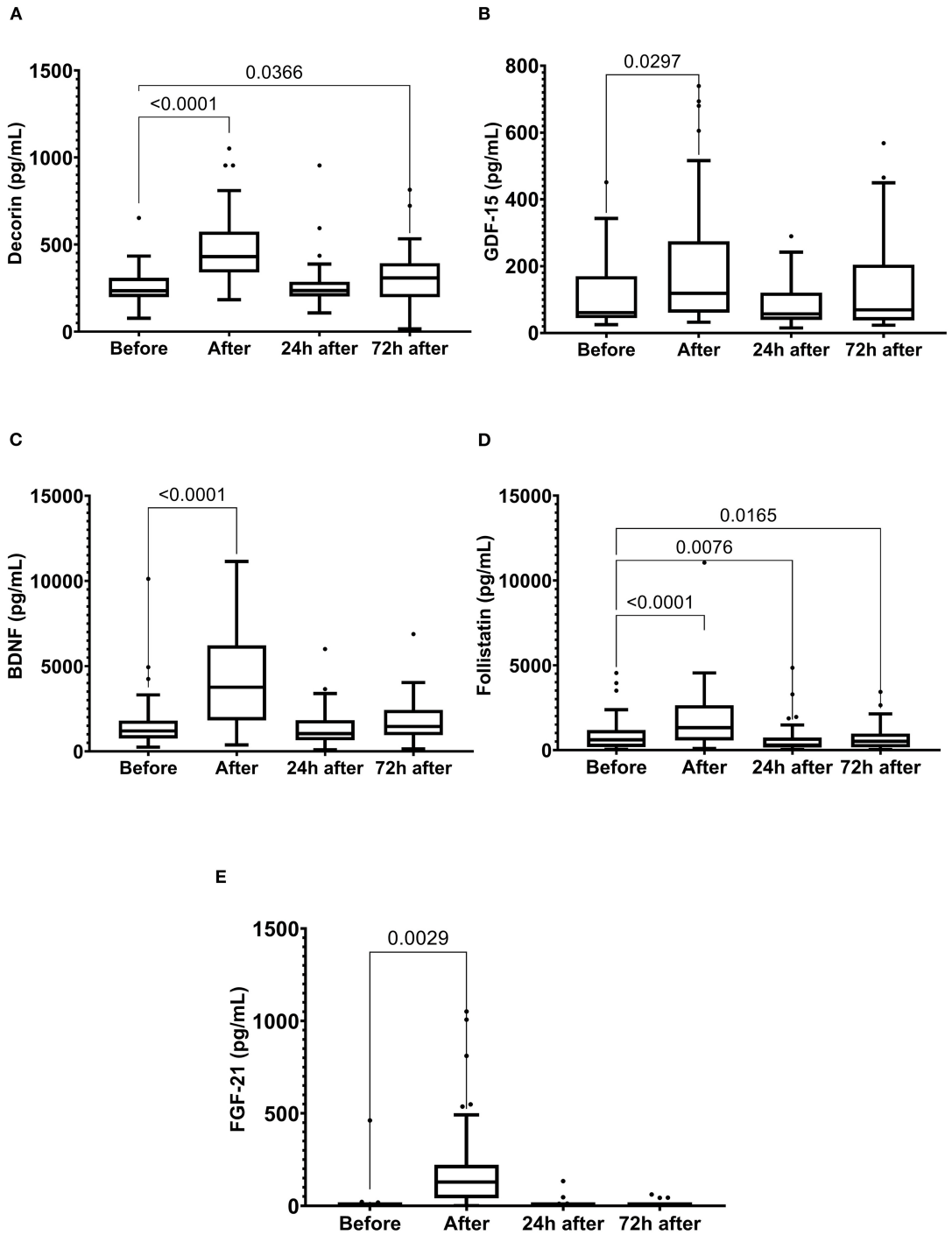

FIGURE 5 | The plasma levels of decorin **(A)**, growth differentiation factor 15 (GDF-15) **(B)**, brain-derived neurotrophic factor (BDNF) **(C)**, follistatin **(D)** fibroblast growth factor 21 (FGF-21) **(E)** immediately after, 24 h after, and 72 h after the race. The values are presented as minimum, maximum, median, and outliers of 48 runners (decorin, GDF-15, BDNF, and Follistatin) or 40 runners (FGF-21) (Box plot, Tukey).

of the innate immune cells is regulated by chemokines, such as IL-8 (Nieman and Mitmesser, 2017; Jones and Davison, 2019; Suzuki et al., 2020). The endurance exercise induces a pro inflammatory response mediated by the neutrophils and pro inflammatory macrophages, M1 in the early hours after exercise, follow an anti-inflammatory compensatory response mediated by M2 macrophages and T cells (Treg and CD8+) leading a period of immunosuppression called "open window"

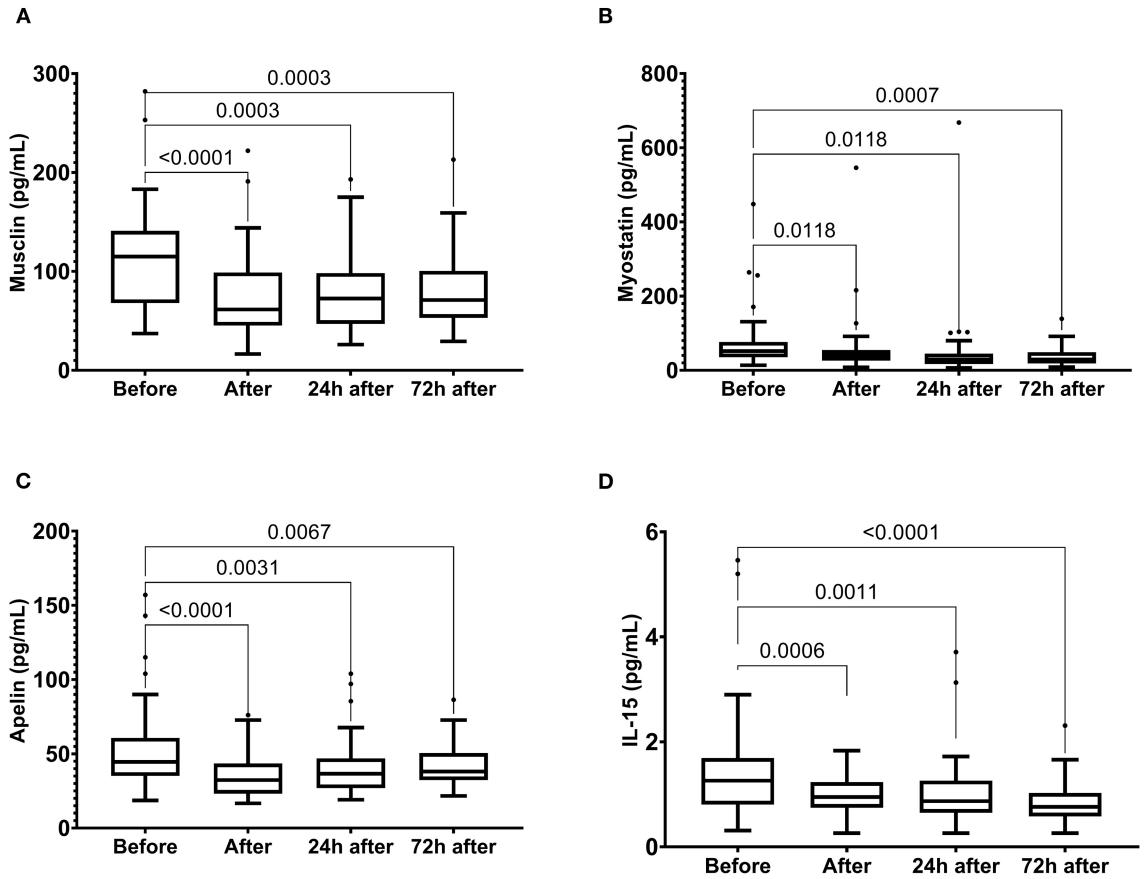

FIGURE 6 | The plasma levels of musclin **(A)**, myostatin **(B)**, apelin **(C)** and IL-15 **(D)** immediately after, 24 h after, and 72 h after the race. The values are presented as minimum, maximum, median, and outliers of 48 runners (Box plot, Tukey).

(Peake et al., 2017; Jones and Davison, 2019; Suzuki et al., 2020).

In this study, the levels of skeletal muscle damage markers were noted to be correlated with the immune changes and inflammatory mediators (IL-10 and TNF-α); however, no association was found between the variations in the levels of skeletal muscle damage markers and myokines. We suggest that regardless of the extent of skeletal muscle damage, other intrinsic muscle factors may modulate the release of myokines, as indicated by an association of free fat mass and/or training experience with the variations in BDNF, GDF-15, irisin, and myostatin levels. We observed an association between age and GDF-15, before and after the race, as proposed by Conte et al. (2020); however, we did not observe a correlation with the total number of leukocytes, neutrophils, and lymphocytes showed in the cyclists after exhaustive exercise (Conte et al., 2020). A GDF-15 is a stress-induced cytokine released from the TGF-β superfamily, in response to the mitochondrial stress and/or inflammatory stress. A GDF-15 has been suggested to induce reorganization and adaptation of systemic metabolism (Chung et al., 2017; Conte et al., 2020; Laurens et al., 2020). Although the contracting skeletal muscle releases GDF15, leading to an increase in the plasma levels, the source of systemic GDF-15 levels after acute exercise remains unclear (Kleinert et al., 2018; Conte et al., 2020).

IL-6, induced by exercise, has a well-known anti-inflammatory effect that modulates the release of IL-10 and IL-1ra. The other paracrine effects of IL-6 on the skeletal muscle include intramuscular lipolysis and improvement of insulin sensitivity and glucose intake (Carey et al., 2006; Wolsk et al., 2010; Laurens et al., 2020). Moreover, the previous studies have observed that an IL-6 induces the following: myogenic differentiation in the C2C12 myoblast cell line and primary human myoblasts, myotube protein synthesis in the C2C12 myoblast cell line, and murine and myoblast proliferation in the human satellite cell proliferation (Pedersen et al., 2001; Gao et al., 2017; Steyn et al., 2019). The studies in animals have elucidated the role of IL-6 in the activation of M2 macrophages, which promotes angiogenesis and damages tissue repair (Pilny et al., 2019), satellite cell proliferation, and myonuclear accretion (Serrano et al., 2008).

In this study, in response to the exercise, the IL-6 expression was positively correlated with that of FGF-21 and follistatin, which are considered hepatokines, rather than the myokines (Domin et al., 2021). Follistatin has myogenic properties that

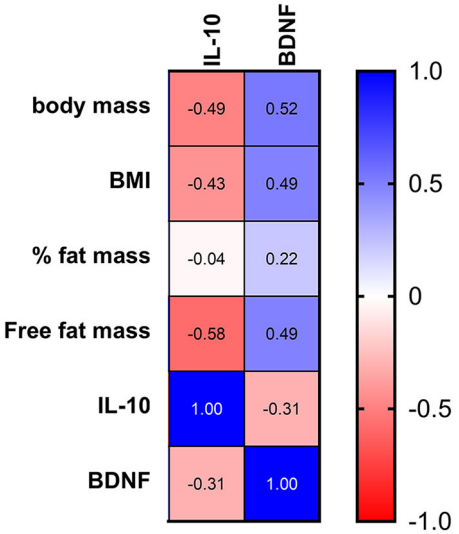

FIGURE 7 | Correlation between the body composition and changes in IL-10 and BDNF after the race. The values are presented as Spearman's r of 43 runners for IL-10 and 38 runners for BDNF. Colormap with range between 1 and −1 and blue for the largest value and red for the smallest value.

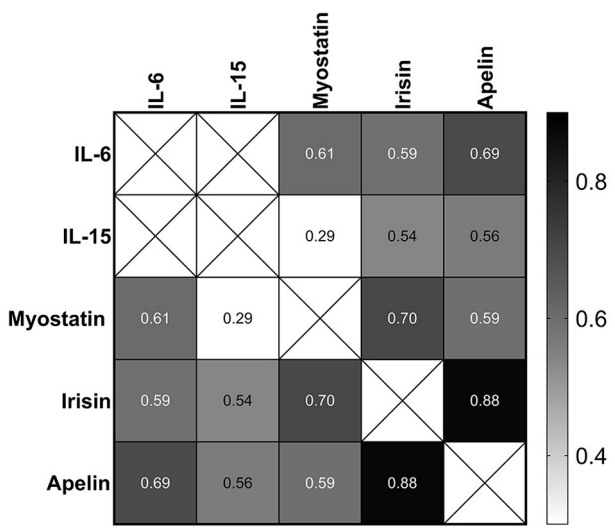

FIGURE 8 | Correlation between the changes in IL-6, IL-15, myostatin, irisin, and apelin 72 h after the race. The values are presented as Spearman's r of 39 runners for irisin, 40 runners for apelin, and 43 runners for IL-6, IL-15, and myostatin. Colormap with range between 0.3 and 0.9 and black for the largest value and white for the smallest value.

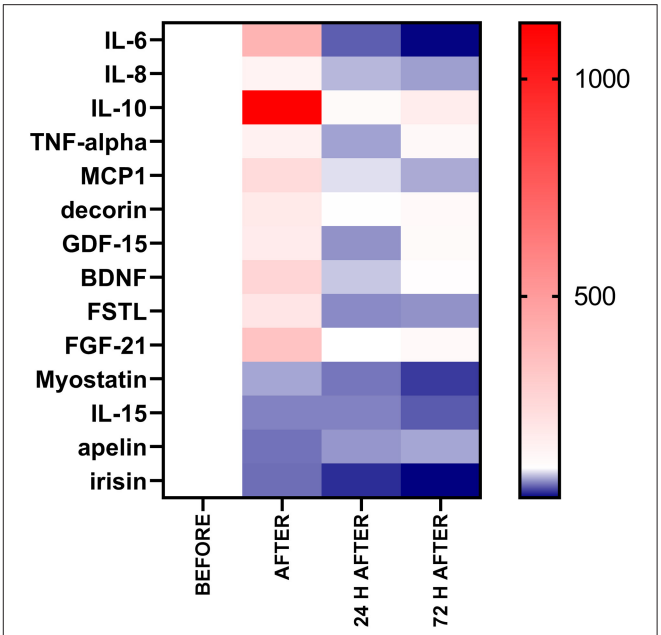

FIGURE 9 | Percentage of baseline value of the exercise-induced cytokines after, 24 h after, and 72 h after. The values are presented as percentage of baseline value (before) compared with after, 24 h after, and 72 h after the race of 38–54 runners. Colormap with range between 1,129 and 37% and red for the largest value, white for the baseline (100%) and blue for the smallest value.

directly inhibit myostatin from binding to the activin IIb receptor and suppression of small mothers against decapentaplegic 3 (Smad3) phosphorylation, consequently increasing the protein synthesis by the mTOR/S6K/S6RP signaling cascade and contributing to skeletal muscle mass (Winbanks et al., 2012; Hoffmann and Weigert, 2017; Lee and Jun, 2019). In our study, the follistatin levels were correlated with those of BDNF, which are responsible for the activation and proliferation of satellite muscle cells after injury (Hoffmann and Weigert, 2017; Lee and Jun, 2019). BDNF had a negative correlation with a myocardial damage marker. A recent review highlighted that BDNF acts on myocardial tissue by decreasing the cardiomyocyte apoptosis and mitochondrial dysfunction and increasing angiogenesis, cardiomyocyte contraction, and calcium cycling *via* tropomyosin-related kinase receptor B (TrkB) signaling pathways (Hang et al., 2021).

Mitochondrial dysfunction and endoplasmic reticulum stress trigger the FGF-21 expression in the skeletal muscles (Tezze et al., 2019). FGF-21 modulates PI3K-AKT signaling, activates ATF4 in skeletal muscle, and is involved in the removal of damaged mitochondria *via* mitophagy and alteration of the type of muscle fiber; thus, it is involved in the regulation of both the muscle mass and function (Hoffmann and Weigert, 2017; Oost et al., 2019; Lee and Jun, 2019). In addition, FGF-21 seems to have metabolic effects similar to those of IL-6 in the skeletal muscle (Tanimura et al., 2016; Struik et al., 2019; Tezze et al., 2019). The FGF-21 response correlated negatively with the decorin and apelin response, indicating that better FGF-21 response may avoid decorin and apelin reduction after the race.

Decorin is a member of the small leucine-rich proteoglycan family of extracellular matrix proteins that interact with the collagen fibers. It has been reported to modulate the proliferation of human skeletal muscle cells and autophagy and the consequent promotion of muscle regeneration (Li

et al., 2007); moreover, it directly inhibits myostatin by activating the SMAD-2/3 complex, thereby reducing the degradation of proteins in the skeletal muscle (El Shafey et al., 2016).

In the recovery period, IL-6 correlated with the myostatin, irisin, and apelin levels. Myostatin acts on the activin receptors (type I and II), promoting the phosphorylation and activation of SMAD proteins. SMAD-2 and SMAD-3 form a complex with SMAD-4, which induces the transcription of catabolic genes. Moreover, myostatin participates in the process of protein degradation through the ubiquitin-proteasome system and autophagy (Hoffmann and Weigert, 2017; Lee and Jun, 2019; Piccirillo, 2019).

The decrease in the levels of other well-known myokines that promote muscle regeneration and modulate autophagy, such as IL-15, apelin, and musclin, indicate that these molecules do not play decisive roles in the repair and regeneration after endurance exercise-mediated muscle damage. IL-15 is a myokine whose levels affect the differentiation of myoblasts, and this myokine modulates autophagy (Hoffmann and Weigert, 2017; Lee and Jun, 2019; Piccirillo, 2019; Trovato et al., 2019; Pesce et al., 2020); moreover, IL-15 is associated with the vascular smooth muscle cell proliferation, stem cell proliferation and differentiation, mitochondriogenesis, autophagy, and anti-inflammatory properties in skeletal muscle (Mughal and O'Rourke, 2018; Vinel et al., 2018).

The exercise-induced inflammatory mediators (IL-10 and IL-8) correlated with the changes in musclin which contains a region homologous to the members of the natriuretic peptide (NP) family. Musclin seems to promote the skeletal muscle oxidative capacity by mitochondrial biogenesis (Subbotina et al., 2015). In contrast to the studies in animals (Farrash et al., 2021), our data showed a decrease of musclin after acute exercise.

Daily dietary intake and food intake during the competition may affect the inflammatory response (Nieman and Mitmesser, 2017). A previous study of our group demonstrated the association of energy, electrolyte, and carbohydrate intakes and inflammatory response after endurance exercise (Passos et al., 2019). The limitation of this study was that it did not assess the role of food intake during the marathon race. Further studies are needed to evaluate the role of daily dietary intake, nutritional supplements, and food intake during the race on the exercise-induced cytokines.

Based on the results of the current study, we conclude that the endurance exercises, such as a marathon could alter the levels of inflammatory markers, and the interaction between the peptides shows that an increase in GDF-15, BDNF, follistatin, decorin, and FGF-21 expression, owing to the decrease in myostatin levels during the recovery period, appears to contribute to the muscle repair and regeneration after a long and high-intensity race. In addition, the regeneration of exercise-induced muscle damage involves the functioning of classical inflammatory mediators and myokines, whose functions include mediation of angiogenesis (IL-6), myogenesis (IL-6 and follistatin), mytophagia (FGF-21), and satellite cell activation (BDNF), in addition to the downregulation of protein (follistatin and decorin) degradative pathways. Additionally, further studies should be carried out to verify the role of BDNF levels in myocardial damage-induced by the race. Furthermore, IL-6 modulates the anti-inflammatory and metabolic responses, and it is associated with the levels of myokines (follistatin, FGF-21, myostatin, apelin, and irisin) that are involved in the muscle repair. The discovery of plasmatic myokines involved in the muscle damage and repair may help to elucidate the endurance exercise muscle adaptations and may yield potential molecular therapeutic targets to treat the myopathies that involve mitochondrial dysfunctions.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Dante Pazzanese Institute of Cardiology, Brazil (Permit Number: 979/2010). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CS carried out the data collection, acquisition analysis, and data interpretation. AS carried out the data collection, participated in its design, and helped draft the manuscript. BM performed acquisition analysis and data interpretation and helped draft the manuscript. JM and RM were responsible for the data collection. HB and HS participated in the experimental design, acquisition analysis, and data interpretation, as well as helped to draft the manuscript. MC-B conceived the study, participated in its design and coordination, helped to perform the statistical analysis, and drafted the manuscript. All authors have read, revised, and approved the final version of the manuscript and agree with the order of presentation of the authors.

FUNDING

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) [grant number 2018/26269-0].

ACKNOWLEDGMENTS

The authors are grateful to the São Paulo International Marathon Organization (Yescom and IDEEA Institute) for their assistance and support in the São Paulo International Marathon; moreover, the authors thank AFIP for their technical assistance.

REFERENCES

Bay, M. L., and Pedersen, B. K. (2020). Muscle-organ crosstalk: focus on immunometabolism. *Front. Physiol.* 11:567881. doi: 10.3389/fphys.2020.567881

Carey, A. L., Steinberg, G. R., Macaulay, S. L., Thomas, W. G., Holmes, A. G., Ramm, G., et al. (2006). Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes* 55, 2688–2697. doi: 10.2337/db05-1404

Chung, H. K., Ryu, D., Kim, K. S., Chang, J. Y., Kim, Y. K., Yi, H. S., et al. (2017). Growth differentiation factor 15 is a myomitokine governing systemic energy homeostasis. *J. Cell Biol.* 216, 149–165. doi: 10.1083/jcb.201607110

Conte, M., Martucci, M., Mosconi, G., Chiariello, A., Cappuccilli, M., Totti, V., et al. (2020). GDF15 plasma level is inversely associated with level of physical activity and correlates with markers of inflammation and muscle weakness. *Front. Immunol.* 11:915. doi: 10.3389/fimmu.2020.00915

Domin, R., Dadej, D., Pytka, M., Zybek-Kocik, A., Ruchała, M., and Guzik, P. (2021). Effect of various exercise regimens on selected exercise-induced cytokines in healthy people. *Int. J. Environ. Res. Public Health* 18:1261. doi: 10.3390/ijerph18031261

El Shafey, N., Guesnon, M., Simon, F., Deprez, E., Cosette, J., Stockholm, D., et al. (2016). Inhibition of the myostatin/Smad signaling pathway by short decorin-derived peptides. *Exp. Cell Res.* 341, 187–195. doi: 10.1016/j.yexcr.2016.01.019

Farrash, W. F., Phillips, B. E., Britton, S. L., Qi, N., Koch, L. G., Wilkinson, D. J., et al. (2021). myokine responses to exercise in a rat model of low/high adaptive potential. *Front. Endocrinol.* 12:645881. doi: 10.3389/fendo.2021.645881

Gao, S., Durstine, J. L., Koh, H. J., Carver, W. E., Frizzell, N., and Carson, J. A. (2017). Acute myotube protein synthesis regulation by IL-6-related cytokines. American journal of physiology. *Cell Physiol.* 313, C487–C500. doi: 10.1152/ajpcell.00112.2017

Gonzalez-Gil, A. M., and Elizondo-Montemayor, L. (2020). The role of exercise in the interplay between myokines, hepatokines, osteokines, adipokines, and modulation of inflammation for energy substrate redistribution and fat mass loss: a review. *Nutrients* 12:1899. doi: 10.3390/nu12061899

Hang, P. Z., Zhu, H., Li, P. F., Liu, J., Ge, F. Q., Zhao, J., et al. (2021). The emerging role of BDNF/TrkB signaling in cardiovascular diseases. *Life* 11:70. doi: 10.3390/life11010070

Hody, S., Croisier, J. L., Bury, T., Rogister, B., and Leprince, P. (2019). Eccentric muscle contractions: risks and benefits. *Front Physiol.* 10:536. doi: 10.3389/fphys.2019.00536

Hoffmann, C., and Weigert, C. (2017). Skeletal muscle as an endocrine organ: the role of myokines in exercise adaptations. *Cold Spring Harbor Perspect. Med.* 7:a029793. doi: 10.1101/cshperspect.a029793

Jones, A. W., and Davison, G. (2019). Exercise, immunity, and illness. *Muscle Exerc. Physiol.* 2019, 317–344. doi: 10.1016/B978-0-12-814593-7.00015-3

Kleinert, M., Clemmensen, C., Sjøberg, K. A., Carl, C. S., Jeppesen, J. F., Wojtaszewski, J. F. P., et al. (2018). Exercise increases circulating GDF15 in humans. *Mol. Metab.* 9, 187–191. doi: 10.1016/j.molmet.2017.12.016

Laurens, C., Bergouignan, A., and Moro, C. (2020). Exercise-released myokines in the control of energy metabolism. *Front. Physiol.* 11:91. doi: 10.3389/fphys.2020.00091

Lee, J. H., and Jun, H. S. (2019). Role of myokines in regulating skeletal muscle mass and function. *Front. Physiol.* 10:42. doi: 10.3389/fphys.2019.00042

Li, Y., Li, J., Zhu, J., Sun, B., Branca, M., Tang, Y., et al. (2007). Decorin gene transfer promotes muscle cell differentiation and muscle regeneration. *Mol. Ther.* 15, 1616–1622. doi: 10.1038/sj.mt.6300250

Mughal, A., and O'Rourke, S. T. (2018). Vascular effects of apelin: mechanisms and therapeutic potential. *Pharmacol. Ther.* 190, 139–147. doi: 10.1016/j.pharmthera.2018.05.013

Nieman, D. C., and Mitmesser, S. H. (2017). potential impact of nutrition on immune system recovery from heavy exertion: a metabolomics perspective. *Nutrients* 9:513. doi: 10.3390/nu9050513

Oost, L. J., Kustermann, M., Armani, A., Blaauw, B., and Romanello, V. (2019). Fibroblast growth factor 21 controls mitophagy and muscle mass. *J. Cachexia Sarcopenia Muscle.* 10, 630–642. doi: 10.1002/jcsm.12409

Passos, B. N., Lima, M. C., Sierra, A., Oliveira, R. A., Maciel, J., Manoel, R., et al. (2019). Association of daily dietary intake and inflammation induced by marathon race. *Mediat. Inflamm.* 2019:1537274. doi: 10.1155/2019/1537274

Peake, J. M., Della Gatta, P., Suzuki, K., and Nieman, D. C. (2015). Cytokine expression and secretion by skeletal muscle cells: regulatory mechanisms and exercise effects. *Exerc. Immunol. Rev.* 21, 8–25.

Peake, J. M., Neubauer, O., Della Gatta, P. A., and Nosaka, K. (2017). Muscle damage and inflammation during recovery from exercise. *J. Appl. Physiol.* 122, 559–570. doi: 10.1152/japplphysiol.00971.2016

Pedersen, B. K., Steensberg, A., and Schjerling, P. (2001). Muscle-derived interleukin-6: possible biological effects. *J. Physiol.* 536, 329–337. doi: 10.1111/j.1469-7793.2001.0329c.xd

Pesce, M., Ballerini, P., Paolucci, T., Puca, I., Farzaei, M. H., and Patruno, A. (2020). Irisin and Autophagy: First Update. *Int. J. Mol. Sci.* 21:7587. doi: 10.3390/ijms21207587

Piccirillo, R. (2019). Exercise-induced myokines with therapeutic potential for muscle wasting. *Front. Physiol.* 10:287. doi: 10.3389/fphys.2019.00287

Pilny, E., Smolarczyk, R., Jarosz-Biej, M., Hadyk, A., Skorupa, A., Ciszek, M., et al. (2019). Human ADSC xenograft through IL-6 secretion activates M2 macrophages responsible for the repair of damaged muscle tissue. *Stem Cell Res. Ther.* 10:93. doi: 10.1186/s13287-019-1188-y

Safdar, A., and Tarnopolsky, M. A. (2018). Exosomes as mediators of the systemic adaptations to endurance exercise. *Cold Spring Harbor Perspect. Med.* 8:a029827. doi: 10.1101/cshperspect.a029827

Serrano, A. L., Baeza-Raja, B., Perdiguero, E., Jardi, M., and Munoz-Canoves, P. (2008). Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy. *Cell Metab.* 7, 33–44. doi: 10.1016/j.cmet.2007.11.011

Steyn, P. J., Dzobo, K., Smith, R. I., and Myburgh, K. H. (2019). Interleukin-6 induces myogenic differentiation via JAK2-STAT3 signaling in mouse C2C12 myoblast cell line and primary human myoblasts. *Int. J. Mol. Sci.* 20:5273. doi: 10.3390/ijms20215273

Struik, D., Dommerholt, M. B., and Jonker, J. W. (2019). Fibroblast growth factors in control of lipid metabolism: from biological function to clinical application. *Curr. Opin. Lipidol.* 30, 235–243. doi: 10.1097/MOL.0000000000000599

Subbotina, E., Sierra, A., Zhu, Z., Gao, Z., Koganti, S. R., Reyes, S., et al. (2015). Musclin is an activity-stimulated myokine that enhances physical endurance. *Proc. Natl. Acad. Sci. U.S.A.* 112, 16042–16047. doi: 10.1073/pnas.1514250112

Suzuki, K., Tominaga, T., Ruhee, R. T., and Ma, S. (2020). characterization and modulation of systemic inflammatory response to exhaustive exercise in relation to oxidative stress. *Antioxidants* 9:401. doi: 10.3390/antiox9050401

Tanimura, Y., Aoi, W., Takanami, Y., Kawai, Y., Mizushima, K., Naito, Y., et al. (2016). Acute exercise increases fibroblast growth factor 21 in metabolic organs and circulation. *Physiol. Rep.* 4:e12828. doi: 10.14814/phy2.12828

Tezze, C., Romanello, V., and Sandri, M. (2019). FGF21 as modulator of metabolism in health and disease. *Front. Physiol.* 10:419. doi: 10.3389/fphys.2019.00419

Trovato, E., Di Felice, V., and Barone, R. (2019). Extracellular vesicles: delivery vehicles of myokines. *Front. Physiol.* 10:522. doi: 10.3389/fphys.2019.00522

Vinel, C., Lukjanenko, L., Batut, A., Deleruyelle, S., Pradere, J. P., Le Gonidec, S., Dortignac, A., Geoffre, N., Pereira, O., Karaz, S., et al. (2018). The exerkine apelin reverses age-associated sarcopenia. *Nat. Med.* 24, 1360–1371. doi: 10.1038/s41591-018-0131-6

Whitham, M., and Febbraio, M. A. (2016). The ever-expanding myokinome: discovery challenges and therapeutic implications. *Nat. Rev. Drug Discov.* 15, 719–729. doi: 10.1038/nrd.2016.153

Winbanks, C. E., Weeks, K. L., Thomson, R. E., Sepulveda, P. V., Beyer, C., Qian, H., et al. (2012). Follistatin-mediated skeletal muscle hypertrophy is regulated by Smad3 and mTOR independently of myostatin. *J. Cell. Biol.* 197, 997–1008. doi: 10.1083/jcb.201109091

Wolsk, E., Mygind, H., Grøndahl, T. S., Pedersen, B. K., and van Hall, G. (2010). IL-6 selectively stimulates fat metabolism in human skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 299, E832–E840. doi: 10.1152/ajpendo.00328.2010

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frontiers in Physiology

ORIGINAL RESEARCH
published: 11 November 2021
doi: 10.3389/fphys.2021.739485

Exercise Enhanced Cardiac Function in Mice With Radiation-Induced Heart Disease *via* the FNDC5/Irisin-Dependent Mitochondrial Turnover Pathway

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: 11 July 2021
Accepted: 21 October 2021
Published: 11 November 2021

Citation:
He W, Tang Y, Li C, Zhang X, Huang S, Tan B and Yang Z (2021) Exercise Enhanced Cardiac Function in Mice With Radiation-Induced Heart Disease via the FNDC5/Irisin-Dependent Mitochondrial Turnover Pathway. Front. Physiol. 12:739485. doi: 10.3389/fphys.2021.739485

Background: Despite the development of radiation therapy (RT) techniques, concern regarding the serious and irreversible heart injury induced by RT has grown due to the lack of early intervention measures. Although exercise can act as an effective and economic nonpharmacologic strategy to combat fatigue and improve quality of life for cancer survivors, limited data on its application in radiation-induced heart disease (RIHD) and the underlying molecular mechanism are available.

Methods: Fifteen young adult male mice were enrolled in this study and divided into 3 groups (including exercised RIHD group, sedentary RIHD group, and controls; $n = 5$ samples/group). While the mice in the control group were kept in cages without irradiation, those in the exercised RIHD group underwent 3 weeks of aerobic exercise on the treadmill after radiotherapy. At the end of the 3rd week following RT, FNDC5/irisin expression, cardiac function, aerobic fitness, cardiomyocyte apoptosis, mitochondrial function, and mitochondrial turnover in the myocardium were assessed to identify the protective role of exercise in RIHD and investigate the potential mechanism.

Results: While sedentary RIHD group had impaired cardiac function and aerobic fitness than controls, the exercised RIHD mice had improved cardiac function and aerobic fitness, elevated ATP production and the mitochondrial protein content, decreased mitochondrial length, and increased formation of mitophagosomes compared with sedentary RIHD mice. These changes were accompanied by the elevated expression of FNDC5/irisin, a fission marker (DRP1) and mitophagy markers (PINK1 and LC3B) in exercised RIHD group than that of sedentary RIHD group, but the expression of biogenesis (TFAM) and fusion (MFN2) markers was not significantly changed.

Conclusion: Exercise could enhance cardiac function and aerobic fitness in RIHD mice partly through an autocrine mechanism *via* FNDC5/irisin, in which autophagy was

selectively activated, suggesting that FNDC5/irisin may act as an intervening target to prevent the development of RIHD.

Keywords: FNDC5, irisin, radiation therapy, heart, aerobic exercise

BACKGROUND

As radiation therapy has been widely applied in thoracic tumor treatment, radiation-induced heart injury, which results in irreversible myocardial fibrosis and heart failure, has also attracted increased attention. The NRG oncology RTOG0617 trial reported that cardiac toxicity may impede the benefit of high-dose radiation therapy (RT) on overall survival (OS) in patients with non-small-cell lung cancer (Bradley et al., 2015). After long-term follow-up in the same cohort, the latest data further support the close relationship between heart V5 and OS, which suggests the potential damage of cardiac toxicity on the outcome after RT (Bradley et al., 2020). A linear correlation between the mean dose to the whole heart and cardiovascular risk was also observed in patients with breast cancer who received RT, without an apparent risk threshold remaining (McGale et al., 2011). Despite investigating RT techniques that minimize radiation exposure to the heart, early identification of and interventions for these complications remain to be reported.

Recent views have extended the cardiovascular toxicity induced by tumor treatment to the cardiovascular-skeletal muscle axis characterized by impaired cardiorespiratory fitness (CRF). The role of exercise as an effective nonpharmacological approach in controlling cardiovascular risk factors, halting the progression of cardiovascular disease and improving outcomes, has been widely accepted and limited, but growing data have supported the benefits of aerobic exercise in improving CRF and reducing cardiovascular risk in cancer survivors to relieve the "whole-body" damage caused by tumor treatment (Bhattacharya and Asaithamby, 2016), implying the potential of exercise in intervening radiation-induced heart disease (RIHD).

Irisin is an exercise-induced myokine that is cleaved from the transmembrane protein fibronectin type III (FNIII) domain-containing protein 5 (FNDC5) expressed in myocytes and may link the cardiovascular-skeletal muscle axis (Ma et al., 2021). Despite the role of FNDC5 as the precursor of irisin, FNDC5 could also act as the receptor of irisin based on its fibronectin type III domain containing the Arg-Gly-Asp (RGD) sequence (Main et al., 1992; Teufel et al., 2002; Erickson, 2013), which might mediate the autocrine or endocrine action of irisin. As cardiac damage induced by ionizing radiation often converges on mitochondria through DNA damage which is susceptible to ionizing radiation (Seol et al., 2012), the positive feedback of prolonged mitochondrial impairment and excessive ROS production may account for the long-term adverse effects following RT (Yahyapour et al., 2017; Schofield and Schafer, 2021). Irisin has recently been reported to be involved in the differentiation of mouse embryonic stem cells by promoting mitochondrial integrity (Nazem et al., 2017). Additionally, irisin could act as an oxidative stress scavenger and alleviate doxorubicin-induced cardiotoxicity (Liu et al., 2019; Zhang et al., 2019). However, the regulatory role of exercise-activated FNDC5/irisin in halting the accumulated RT-induced mitochondrial damage to cardiomyocytes remains unknown. Therefore, we aimed to investigate the protective role of aerobic exercise in RIHD and its potential regulatory role in mitochondrial function *via* the FNDC5/irisin pathway.

MATERIALS AND METHODS

Animals

Fifteen young adult male C57BL/6 mice (6 weeks old, weighing 12–15 g) were purchased from the animal laboratory of Chongqing Medical University. The posterior evaluation of sample size was conducted by one-way analysis of variance F test using PASS 15.0.5. The total sample size of 6 mice ($n = 3$ groups) has achieved 100% power to detect the difference of means of irisin levels [K(Means Multiplier) = 1, Effect size $f = 7.68$, 7.46, 4.51, α err prob. = 0.05, Means = 69.32, 25.67, 53.68, Standard Deviation = 2.35, 2.42, 4.00] among three groups. As 15 mice were enrolled in our study, the 100% power has been achieved to detect the difference among the means using an F test with a 0.05 significance level. To adjust the effect of confounders, such as feeding conditions and environmental factors, five mice were included per group, considering that five mice in a group were kept in the same cage with a controlled room temperature of 22 ± 2°C under a 12-h light/dark cycle and given free access to water and food. Since estrogen is a protective factor against the risk of cardiovascular disease and heart disease, which has rarely occurred in female populations in earlier life stages (Iorga et al., 2017), we excluded female mice from the current study. In order to ensure the replicability, five samples/group was set to evaluate the effect of exercise on body weight, grip strength, cardiac function, aerobic fitness, and mitochondrial function and three samples/group with three duplicates/sample was set to evaluate the effect of exercise on the expressions of FNDC5/irisin and mitochondrial turnover markers.

All experimental procedures were conducted according to the ARRIVE guidelines and were approved by the Animal Care and Use Committee of Chongqing Medical University No. 2021053.

The mice were randomly divided into three groups: the control group ($n = 5$), sedentary RIHD group ($n = 5$), and exercised RIHD group ($n = 5$). While the mice in the control group were kept in cages without irradiation, those in the exercised RIHD group underwent aerobic exercise intervention. All mice were sacrificed on the 21st day after exposure of the heart to X-ray irradiation. The mice in the sedentary

RIHD group remained free within the cage and were sacrificed 21 days after irradiation.

Establishment of the RIHD Model

A single session of radiation exposure was conducted in this study, which has been previously reported (Kruse et al., 2003). The precordial area of each mouse was exposed to X-ray irradiation individually to establish a murine RIHD model. After all mice had been anesthetized with isoflurane anesthesia (2%) using a mask, the hair on the chest was removed, and irradiation was applied with a 6 MV X-ray beam energy at a dose of 20 Gy/1 Fx with a 100-cm source surface distance in a 1 × 1-cm radiation field of the precordial area.

Exercise Protocol

The moderate aerobic exercise protocol used with the exercised mice was performed on an animal treadmill (Zhongshi, Inc.), which has been described by our previous study (Wuyang et al., 2020). The angle of inclination of the treadmill is 0°. The period of the exercise trial is 3 weeks. In the first week, an adaptation protocol was conducted (5 days/week, 6 m/min for 30 min per day) to improve the reliance of the mice in following the protocol. During the remaining 2 weeks, a moderate exercise protocol with moderate intensity was conducted [5 days/week, 75% VO2 max (10 m/min)] for 1 h per day after the warm-up exercise (4 m/min for 2 min; Fernando et al., 1993). Once the mice were exhausted and could not reach the belt speed, they were allowed to rest for 30–60 min, after which the protocols continued. The period of time to exhaustion was recorded at the end of each week.

Echocardiography

Echocardiography (ESAOTE S.p.A., SL3116, Italy) was used to measure the cardiac function of all the subjects on the 21st day before euthanasia as previously described. Afterward, each mouse was anesthetized using isoflurane, and the hair on the chest was removed using a depilatory cream. The lower left ventricular end-diastolic dimension (LVEDD) and systolic left ventricular dimension (SLVD) were recorded *via* short-axis M-mode. The ejection fraction (EF) was calculated using the equation EF = stroke volume (SV)/end-diastolic volume (VD) × 100%.

Assessment of Grip Strength

An electronic grip strength meter (cat. 47200, Ugo Basil) was used to assess the grip strength of the forelimbs of all the mice at the end of each week. With mice fixed on a fence, the maximal grip strength was measured by slowly pulling at the base of the mouse tails. The procedure was repeated three times, and the highest value was recorded.

Histological Analysis

After euthanasia, the blood was drained, and the tissue of the left ventricle without the septum was dissected and washed with ice-cold saline solution. The cardiac tissues were embedded in paraffin after fixation in paraformaldehyde fixative (4% paraformaldehyde) and then sliced into 5-μm-thick samples for further analysis. The method was performed according to a previously described protocol (Zeng, 2016). Hematoxylin and eosin (HE) and Masson staining were conducted according to a previously described protocol. The sections were analyzed by two independent single-blinded investigators under bright field microscopy (Thermo Scientific) and quantified using Image-Pro Plus 6.0.

Assessment of Apoptosis

Cardiomyocyte apoptosis was assessed using a Cell Death Detection kit (4AF488 TUNEL assay, cat. No. FXP142-050, 4A Biotech, Inc.). The mean number of TUNEL-positive cells was assessed by analyzing 5 randomly chosen regions with Image-Pro Plus 6.0.

Transmission Electron Microscopy

A 1-mm^3 myocardium tissue block was fixed, dehydrated, and dyed with uranyl acetate and lead citrate as described previously (Cullen et al., 2010). Two experienced single-blinded investigators observed the samples by transmission electron microscopy (Hitachi7700, Japan), and ten random fields of each section were analyzed.

Mitochondrial Function

Preparation of Mitochondria From Skeletal Muscle

Fresh myocardium tissues were homogenized with a glass homogenizer on ice within 1 h after euthanasia. Isolation buffer (c3606, Beyotime, Shanghai, China) included in the tissue mitochondria isolation kit was used to isolate mitochondria from cardiac tissue. According to the manufacturer's instructions for the kit, isolation buffer was added to homogenized tissue after the cardiac tissue was dissected and washed with ice-cold PBS solution and trypsin solution. Then, the supernatant was collected after the homogenate was centrifuged (600 × g/min for 5 min, 4°C) in a low-temperature centrifuge. After the supernatant was further centrifuged (11,000 × g/min for 10 min, 4°C), the mitochondria (sediment) was isolated. The mitochondrial pellet was resuspended in mitochondrial isolation buffer (Beyotime, Shanghai, China) as previously described (Ouyang et al., 2016).

Mitochondrial Protein Content

The mitochondrial protein content was measured with an enhanced BCA protein assay (Beyotime, China, P0010S) according to a standardized protocol.

Mitochondrial Membrane Potential (ΔΨm) Measurement

The mitochondrial pellet was stained with the JC-1 probe using a ΔΨm assay kit with JC-1 (Beyotime, China, c2006). A modular multitechnology microplate reader (Thermo Scientific Varioskan LUX) was used to assess the absorption of monomers

(fluorescence excitation was set at 490 nm, and fluorescence excitation was set at 530 nm) and J-aggregates (fluorescence excitation was set at 525 nm, and fluorescence excitation was set at 590 nm) according to the manufacturer's protocol. Differences in ΔΨm among groups were reflected by the ratio of J-aggregates/monomer.

ATP Assessment

After homogenization of fresh myocardial tissue on ice, the supernatant was collected, and adenosine 5′-triphosphate (ATP) levels were measured with a modular multitechnology microplate reader (Thermo Scientific™ Varioskan™ LUX) according to the guidelines of an ATP assay kit (Beyotime, China, S0026). The ATP content was normalized to the ATP protein concentration, which was measured with an enhanced BCA Protein Assay kit (Beyotime, China, P0010S).

Real-Time PCR

The PCR primers specific for FNDC5, mitophagy markers (PINK1, PARKIN, and LC3B), mitochondrial fission markers (DRP1 and FIS1), a mitochondrial fusion marker (MFN2), and a mitochondrial biogenesis marker (TFAM) are listed in **Table 1**. RNA was extracted from myocardial tissue using TRIzol reagent (TaKaRa, Inc.). The PrimeScript RT Reagent Kit (TaKaRa, Inc.) was used to conduct RNA denaturation and reverse transcription to generate cDNA. Target genes were assessed by quantitative real-time PCR with three duplicates for each sample. The 2-ΔΔCT method was used to analyze the data.

ELISA

The irisin concentration in serum collected before euthanasia was measured *via* ELISA (MB-5653B, MB Biology, China) according to the manufacturer's guidelines.

Statistical Analyses

The normality of continuous variables was tested by the Kolmogorov–Smirnov test. ANOVA was used to compare normally distributed variables (including body weight, grip strength, grip strength/body weight, ejection fraction, transverse diameter of myocardial fiber, mitochondrial protein concentrations, concentrations of ATP, time to exhaustion, and maximal velocity) among groups. Skewed variables (including mRNA expression of FNDC5, PINK1, LC3B, and DRP1) were compared by the Mann–Whitney U test. The data are shown as the mean ± standard deviation (SD) or median with interquartile ranges for quantitative values. Linear relationships between irisin levels and the expression of mitochondrial turnover markers were assessed by Spearman correlation analysis. All data were analyzed using IBM SPSS Statistics version 25.0 and GraphPad Prism 6, and a value of $p < 0.05$ was used to indicate significance. The one-way analysis of variance F test was conducted by PASS 15.0.5 (NCSS, LLC, version2015) to evaluate the sample size and the power of the current study.

RESULTS

The Effect of Exercise on Body Weight and Grip Strength

While there was no significant difference in body weight among the groups at baseline, the body weight of the RIHD mice was significantly lower than that of the controls at the end of the 2nd and 3rd weeks after irradiation (2nd week: F value = 33.22, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 12.26 ± 0.99 vs. 15.77 ± 0.31 g, 95%CI −4.48–−2.54, $p < 0.001$; 3rd week: F value = 40.84, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 12.30 ± 0.98 vs. 17.21 ± 0.91 g, 95%CI −6.10–−3.73, $p < 0.001$), which indicates that RT may have affected murine body weight (**Figure 1A**). As the exercised RIHD group had higher body weight compared with the sedentary group at the end of each week (1st week:14.85(14.49–15.13) vs. 11.52(10.49–12.13) g, $p = 0.035$; 2nd week: F value = 33.22, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 14.82 ± 0.63 vs. 12.26 ± 0.99 g, 95%CI 1.59–3.53, $p < 0.001$; 3rd week: F value = 40.84, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 14.54 ± 0.67 vs. 12.30 ± 0.97 g, 95%CI 1.05–3.42, $p = 0.001$; **Figure 1A**), exercise may impede the decrease in body weight induced by ionizing radiation.

As sedentary RIHD mice had significantly lower grip strength than controls at the end of the 3rd week

TABLE 1 | PCR primer sequences.

Gene name	Sequence: 5'-3'(forward)	Sequence: 3'-5'(reverse)
GAPDH	CCTCGTCCCGTAGACAAAATG	TGAGGTCAATGAAGGGGTCGT
FNDC5	CACCTCAAGGCCAACTCTGC	CATGGTCACCTCATCTTTGTTCTT
PINK1	TGACCCACTGGACACTCGATG	TGGAGGAACCTGCCGAGAT
PARKIN	CCAGCAGTTAAACCCACCTACAA	AATTAAGACATCGTCCCAGCAAG
MFN2	AGATTACGGAGGAAGTGGAAAGG	GCATAGATACAGGAAGAAGGGGC
Drp1	ATTCCATTATCCTCGCCGTCAC	GTTCTGCGCCCATCTGGATC
LC3B	CGTCCTGGACAAGACCAAGTTC	GCAAGCGCCGTCTGATTATC
TFAM	GGCACCGTATTGCGTGAGAC	GGAAAAACACTTCGGAATACAGAC

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; FNDC5, fibronectin type III domain containing 5; PINK1, PTEN induced putative kinase 1; MFN2, Mitofusin-2; Drp1, Dynamin-1-like protein; TFAM, Transcription factor A mitochondria.

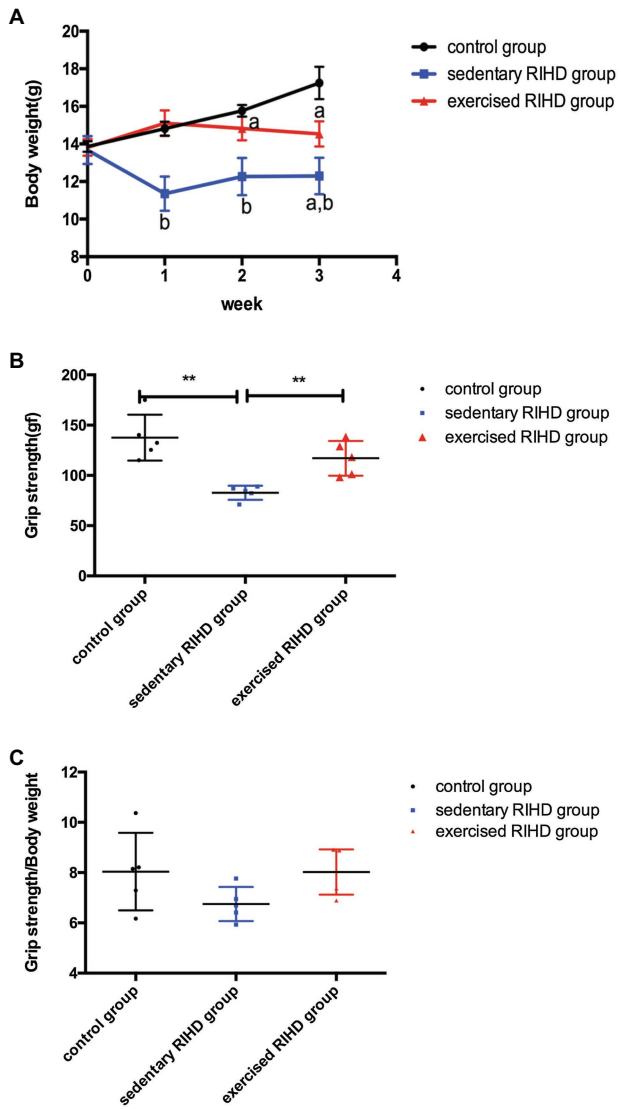

FIGURE 1 | The effect of exercise on body weight and grip strength. **(A)** Body weights of the control group (black line, $n = 5$), sedentary RIHD group (blue line, $n = 5$), and exercised RIHD group (red line, $n = 5$) at different time points. The results are presented as the mean and standard derivation. $p^a < 0.05$ compared with the control group, $p^b < 0.05$ compared with the sedentary RIHD group. Grip strength **(B)** and grip strength/body weight **(C)** at the end of the 3rd week after irradiation were compared by ANOVA. $^{**}p < 0.01$.

(F value = 13.21, degrees of freedom (between groups) =2, degrees of freedom (within groups) =12, 82.72 ± 7.02 vs. 137.60 ± 22.87 gf, 95%CI −57.78–−10.78, $p = 0.008$), exercised RIHD mice had increased grip strength than sedentary RIHD mice (F value = 13.21, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 117.00 ± 17.33 vs. 82.72 ± 7.02 gf, 95%CI 10.78–57.78, $p = 0.008$; **Figure 1B**). However, the difference in relative grip strength (grip strength/body weight) was not significant among the groups after adjustment for body weight (F value = 2.25, degrees of freedom (between groups) =2, degrees of freedom (within groups) = 12, $p = 0.149$; **Figure 1C**).

The Effect of Exercise on Cardiac Function and Exercise Fitness

The EF of mice in the sedentary RIHD group was significantly lower than that of controls (F value = 15.70, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 0.70 ± 0.05 vs. 0.83 ± 0.04, 95%CI −0.18–−0.08, $p < 0.001$), but the exercised RIHD group had comparatively higher EF than the sedentary RIHD group (F value = 15.70, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 0.78 ± 0.03 vs. 0.70 ± 0.05, 95%CI 0.03–0.13, $p = 0.006$; **Figure 2A**). While the sedentary RIHD group had significantly decreased LVEDD compared with the controls (F value = 36.64, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 2.34 ± 0.09 vs. 2.82 ± 0.08 mm, 95%CI −0.61–−0.35, $p < 0.001$), exercised RIHD mice had increased SLVD and LVEDD than that of sedentary RIHD mice (SLVD: 1.60(1.55–1.65) vs. 1.50(1.40–1.50) mm, $p = 0.035$; LVEDD: F value = 36.64, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 2.76 ± 0.11 vs. 2.34 ± 0.09 mm, 95%CI 0.29–0.55, $p < 0.001$; **Figures 2B,C**). These data can support that exercise can improve the recovery of cardiac function following RT.

Considering that aerobic fitness, the time to exhaustion and maximal velocity were significantly decreased in the sedentary RIHD mice compared with the controls (time to exhaustion: F value = 24.36, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 15.79 ± 0.67 vs. 26.94 ± 4.76 min, 95%CI −15.33–−6.98, $p < 0.001$; maximal velocity: F value = 54.24, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 22.60 ± 1.82 vs. 30.80 ± 1.48 m/min, 95%CI −10.84–−5.56, $p < 0.001$). Exercised RIHD group had increased time to exhaustion and maximal velocity than sedentary RIHD group (time to exhaustion: F value = 24.36, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 27.75 ± 2.11 vs. 15.79 ± 0.67 min, 95%CI 7.79–16.14, $p < 0.001$; maximal velocity: F value = 54.24, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 35.00 ± 2.35 vs. 22.60 ± 1.82 m/min, 95%CI 9.76–15.04, $p < 0.001$), which suggests exercise may enhance the aerobic fitness of RIHD mice (**Figures 2D,E**).

Exercise Could Improve Cardiac Myopathy Through the FNDC5/Irisin-Dependent Pathway

The Effect of Exercise on FNDC5/Irisin Expression

The mRNA expression of FNDC5 in the myocardium was decreased in sedentary RIHD mice compared with the controls (F value = 180.68, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 0.35 ± 0.05 vs. 1.00 ± 0, 95%CI −0.73 - −0.58, $p < 0.001$), but exercised RIHD group had increased FNDC5 expression than sedentary RIHD group

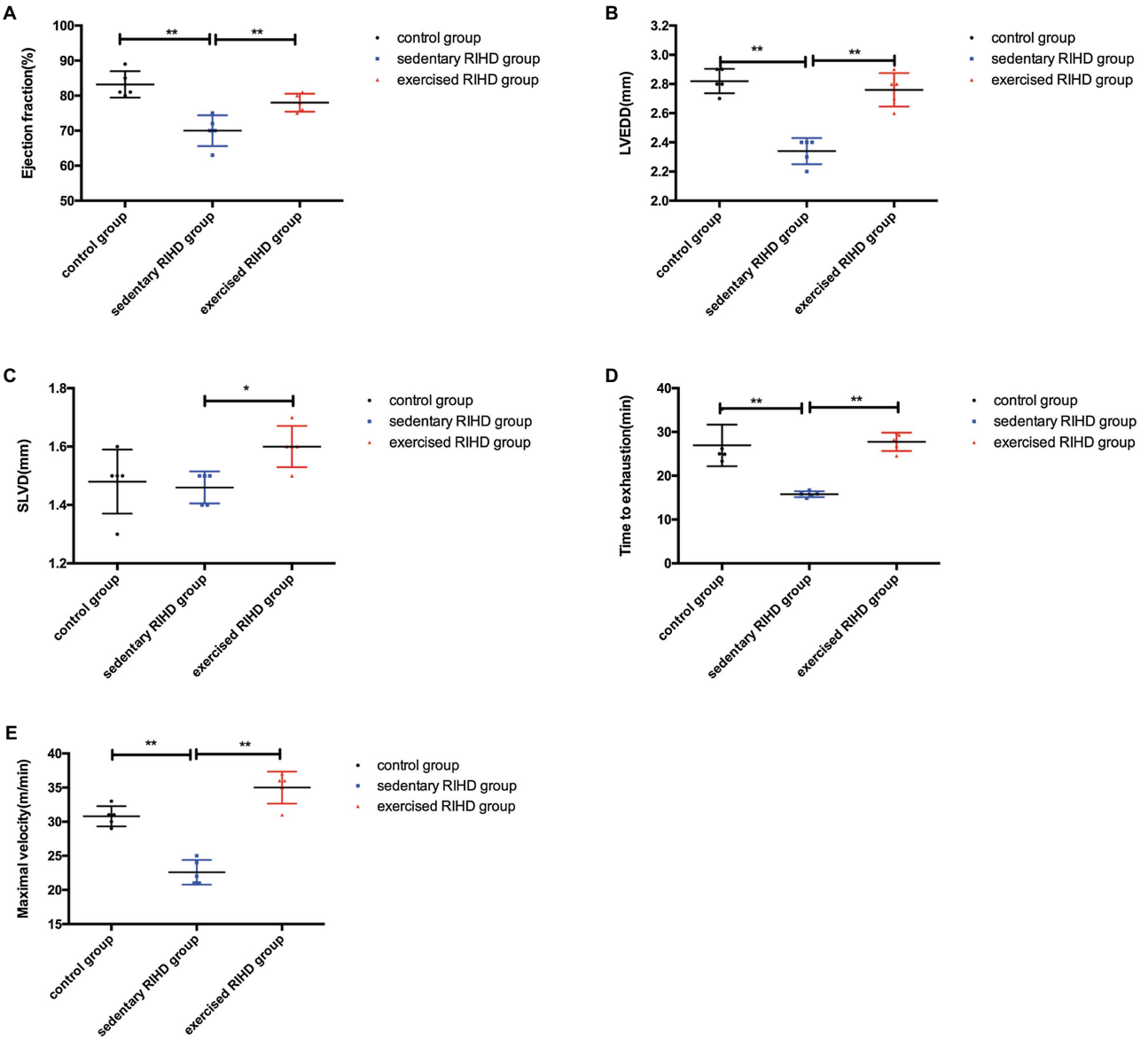

FIGURE 2 | The effect of exercise on cardiac function and aerobic fitness in RIHD mice. Values for EF **(A)** and LVEDD **(B)** among groups were compared by ANOVA. **(C)** The Mann–Whitney U test was used to compare the SLVD among groups. **(D)** Time to exhaustion among the groups was compared by ANOVA. **(E)** Maximal velocity among the three groups was compared by ANOVA. $n = 5$ samples/group. $^{*}p < 0.05$, $^{**}p < 0.01$. LVEDD, lower left ventricular end-diastolic dimension; SLVD, systolic left ventricular dimension; EF, ejection fraction.

(F value = 180.68, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 0.77 ± 0.08 vs. 0.35 ± 0.05, 95%CI 0.35–0.50, $p < 0.001$). Consistent with the alterations in FNDC5 expression, the serum irisin levels were decreased in the sedentary RIHD group compared with the normal controls (F value = 8.323, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 11, 34.47 ± 20.55 vs. 68.90 ± 2.58 ng/ml, 95%CI −53.17–−1.54, $p = 0.002$) and exercised RIHD mice had significantly higher levels of irisin in contrast with sedentary RIHD mice (F value = 8.323, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 11, 53.68 ± 4.00 vs. 34.47 ± 20.55 ng/ml, 95%CI 1.54–36.88, $p = 0.036$; **Figure 3A**).

The Effect of Exercise on Cardiac Myopathy

HE staining showed that sedentary RIHD group presented disarranged myocardial fibers, cardiomyocyte degeneration, nuclear condensation, eosinophilic enhancement, and more inflammatory cell infiltration than the controls. However, exercised RIHD mice had more ordered myocardial fibers with comparatively longer transverse diameters and decreased degeneration, cardiomyocyte nuclear condensation, and

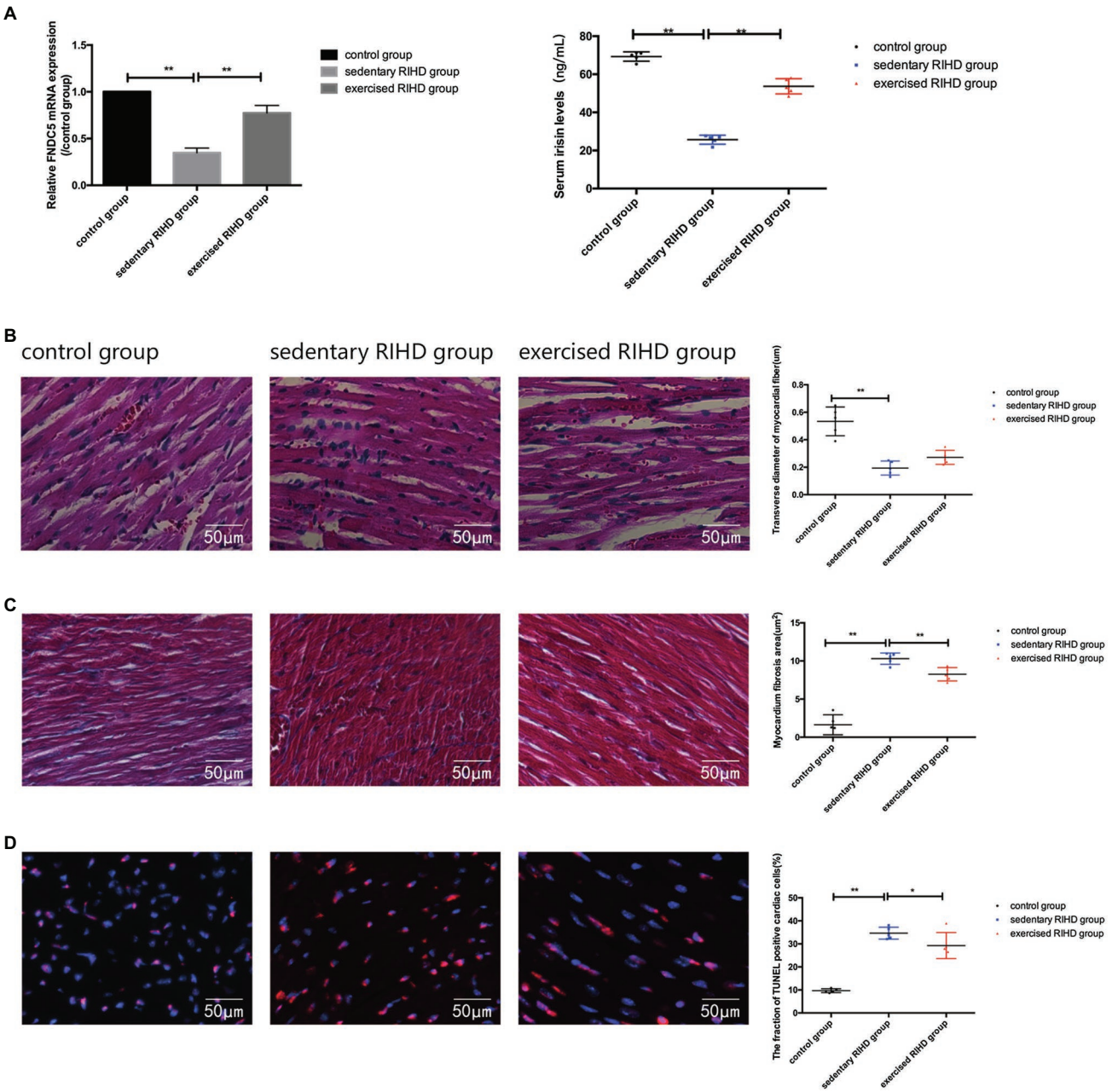

FIGURE 3 | Effect of exercise on FNDC5/irisin expression and cardiac myopathy in RIHD mice. **(A)** Comparison of the relative mRNA expression levels of FNDC5/irisin among the three groups. **(B)** Hematoxylin–eosin staining of the three groups ($n = 3$ samples/group). Magnification, ×400. The transverse diameters of the myocardial fibers between groups were compared by ANOVA. **(C)** Masson staining of the three groups ($n = 3$ samples/group). Magnification, ×400. The quantified myocardial fibrosis areas among the groups were compared by ANOVA. **(D)** TUNEL-positive cells (indicated by the colocalization of red and blue fluorescence) were counted in three groups ($n = 3$ samples/group). Magnification ×400. The fractions of TUNEL-positive cells were compared by ANOVA. $^{*}p < 0.05$, $^{**}p < 0.01$.

inflammatory cell infiltration compared to the sedentary RIHD group (**Figure 3B**).

Masson staining illustrated that the sedentary RIHD group exhibited a significantly higher myocardial fibrosis area than controls (F value = 101.17, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 10.30 ± 0.74 vs. 1.63 ± 1.31 μm^2, 95%CI 7.28–10.06, $p < 0.001$), which was characterized by perivascular fibrosis. Exercise training decreased the myocardial fibrosis area in the RIHD mice (F value = 101.17, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 8.26 ± 0.88 vs. 10.30 ± 0.74 μm^2, 95%CI −3.44–−0.66, $p = 0.007$; **Figure 3C**).

The fraction of TUNEL-positive cardiac cells was significantly higher in the sedentary RIHD group than the controls

(*F* value = 65.60, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 34.64 ± 2.57 vs. 9.68 ± 0.84, 95%CI 19.97–29.96, $p < 0.001$), revealing increased cardiomyocyte apoptosis in response to RT. The exercised RIHD group exhibited a significantly lower fraction of TUNEL-positive myocytes (*F* value = 65.60, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 29.27 ± 5.67 vs. 34.64 ± 2.57, 95%CI −10.37–−0.38, $p = 0.037$), indicating that exercise training may decrease cardiomyocyte apoptosis (**Figure 3D**).

The Effect of Exercise on Mitochondrial Function and Mitochondrial Turnover

Although the sedentary RIHD group had a significantly lower ΔΨm (as indicated by the ratio of JC-1 fluorescence intensity) than the controls (*F* value = 13.07, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 4.12 ± 0.46 vs. 5.22 ± 0.17, 95%CI −1.58–−0.62, $p < 0.001$), there was no significant difference in ΔΨm between the exercised RIHD and sedentary RIHD groups. Interestingly, while sedentary RIHD mice had comparatively lower mitochondrial protein concentrations and a lower ATP content than controls (mitochondrial protein concentrations: *F* value = 65.60, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 0.58 ± 0.05 vs. 0.94 ± 0.15 mg/ml, 95%CI −0.49–−0.23, $p < 0.001$; ATP content: *F* value = 41.62, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 1.60 ± 0.38 vs. 4.92 ± 0.56 nmol/mg, 95%CI −4.12–−2.52, $p < 0.001$), exercised RIHD mice had significantly increased production of mitochondrial proteins and ATP (mitochondrial protein concentrations: F value = 65.60, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 0.75 ± 0.05 vs. 0.58 ± 0.05 mg/ml, 95%CI 0.04–0.30, $p = 0.01$; ATP content: F value = 41.62, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, 2.94 ± 0.74 vs. 1.60 ± 0.38 nmol/mg, 95%CI 0.55–2.14, $p = 0.003$; **Figure 4E**).

In agreement with the alterations in mitochondrial function, the sedentary RIHD mice showed more swollen mitochondria and fewer mitophagosomes in myocardium than controls. Quantitative analysis of the electron microscopy images showed that exercised RIHD mice had a shorter mitochondrial length (*F* value = 40.40, degrees of freedom (between groups) =2, degrees of freedom (within groups) =12, 0.84 ± 0.06 vs. 1.55 ± 0.15 um, 95%CI −0.88–−0.54, $p < 0.001$) and more mitophagosomes ($p = 0.009$) than those in the sedentary RIHD mice (**Figure 4A**), which indicates the enhanced mitochondrial fission and mitophagy in response to exercise. As a result, exercise was found to decrease ultrastructural damage to mitochondria and regulate mitochondrial turnover in RIHD mice.

Although the expression of a mitochondrial biogenesis marker (TFAM1; $p = 0.03$), a mitochondrial fusion marker (MFN2; $p = 0.002$), a mitochondrial fission marker (DRP1; *F* value = 67.99, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, $p < 0.001$), and mitophagy markers (PINK1, PARKIN, LC3B) was significantly decreased in sedentary RIHD mice compared to controls (PINK1: F value = 95.50, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, $p < 0.001$; PARKIN: $p = 0.002$; LC3B: F value = 10.00, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, $p = 0.017$; **Figures 4B–D**), the mRNA expression of DRP1, PINK1, and LC3B was significantly elevated in exercised RIHD mice than that of sedentary RIHD mice (DRP1: F value = 67.99, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, $p < 0.001$; PINK1: F value = 95.50, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, $p < 0.001$; LC3B: F value = 10.00, degrees of freedom (between groups) = 2, degrees of freedom (within groups) = 12, $p = 0.02$), which indicates that exercise may regulate mitochondrial turnover by enhancing mitochondrial fission and mitophagy.

DISCUSSION

The increased cardiovascular risk associated with cancer care is often multifaceted and can be attributed to both impairment induced by tumor therapy and indirect toxic effects in the whole patient. Despite the protective role of aerobic exercise against cardiovascular risk and morbidity caused by anticancer therapy, which has been revealed by a growing amount of data (Codella et al., 2015; Scott et al., 2018a,b), the efficacy and regulatory mechanism of aerobic exercise in RIHD remain unknown. Our study adds to the literature by illustrating that aerobic exercise could promote the recovery of cardiac function and aerobic fitness following RT in RIHD mice. The cardiovascular benefit of aerobic exercise may occur through the regulation of mitochondrial turnover mediated by the FNDC5/irisin pathway.

In our study, aerobic exercise training significantly elevated the body weights of RIHD mice following RT. Since skeletal muscle mass accounts for most of the body mass, we speculate that aerobic exercise maintains body weight mainly by preventing muscle atrophy, consistent with previous data (Guo et al., 2019). Grip strength is a marker reflecting fatigue and frailty status, and aerobic exercise produced increased grip strength at the end of the 3rd week after RT, although the difference was not significant after adjusting for the effect of body weight. These results indicate that exercise may decrease fatigue and frailty following RT partly by increasing skeletal muscle mass. Anticancer therapy produces toxic effects extending to the heart-skeletal muscle axis, leading to impaired CRF and worsening outcomes (Stenehjem et al., 2016; Sweegers et al., 2019). We also confirmed that aerobic exercise strengthened aerobic fitness based on the reduced time to exhaustion and faster maximum velocity in RIHD mice that underwent exercise training.

While the alteration in EF on the 21st day after RT did not cause heart failure, the longer LVEDD in the sedentary RIHD mice suggested impairment of the contractile reserve and LV stiffness at an earlier stage of RIHD. Aerobic exercise can protect cardiac function by elevating both the contractile reserve and systolic function of the left ventricle, which is

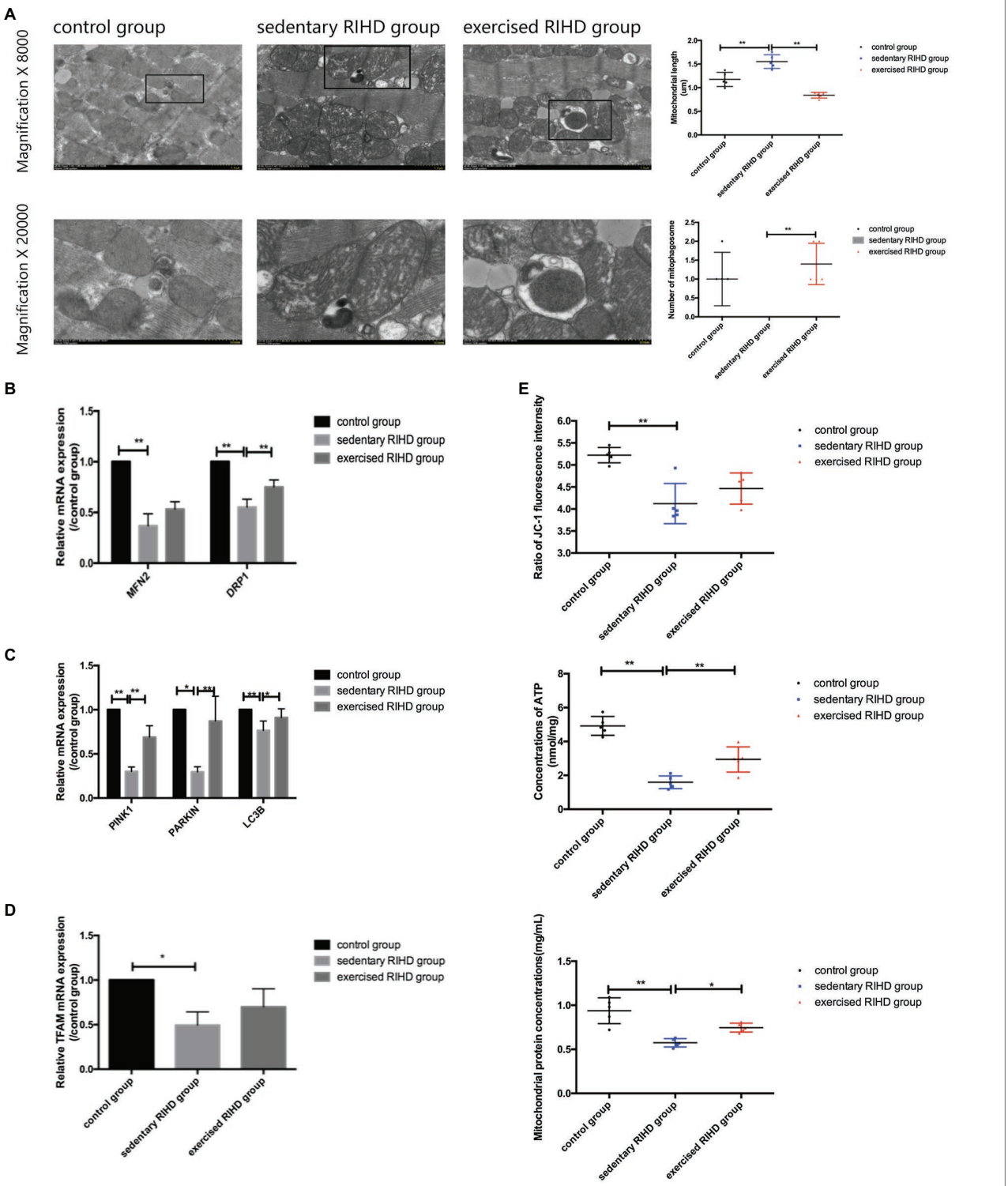

FIGURE 4 | The effect of exercise on mitochondrial function and mitochondrial turnover markers in cardiomyocytes in RIHD mice. **(A)** The formation of mitophagosomes (indicated by the black square) was observed in the control and exercised RIHD groups. The mitochondrial length and number of mitophagosomes were quantified *via* Image-Pro Plus 6.0 ($n=3$ samples/group). ANOVA was used to compare the mitochondrial length among groups, while the number of mitophagosomes was compared using the Mann–Whitney U test. **(B)** Comparison of the relative mRNA expression of mitochondrial dynamics among the groups. **(C)** Comparison of the relative mRNA expression of markers of mitophagy markers in the groups. **(D)** Comparison of the relative mRNA expression of a mitochondrial biogenesis marker among groups. **(E)** The effect of exercise on the mitochondrial function of cardiomyocytes after irradiation. ANOVA was used to compare the mitochondrial membrane potential ($\Delta\Psi m$), ATP production, and mitochondrial protein concentrations among the groups ($n=5$ samples/group). $^{*}p<0.05$, $^{**}p<0.01$.

characterized by improvements in LVEDD, SLVD, and EF. This phenomenon may be further supported by the presence of less damage to cardiomyocytes as assessed by histological characterization, fewer perivascular fibrosis area, and decreased cardiomyocyte apoptosis observed in this study. These changes could slow the progressive process of cardiac dysfunction and remodeling caused by irradiation.

Since the mitochondrial DNA of cardiomyocyte is susceptible to irradiation (Piquereau et al., 2013; Hargitai et al., 2020), the prolonged damage to mitochondrial DNA can cause mitochondrial dysfunction and further disturbed the metabolic homeostasis of cardiomyocyte. Considering that the dynamic balance between mitochondrial biogenesis, fusion, fission, and mitophagy ensures initial mitochondrial quality control (MQC; Ni et al., 2015), the accumulation of abnormal mitochondria observed in myocardium of the sedentary RIHD group may support the disturbed mitochondrial dynamics in response to RT (Martinet et al., 2002; Puente et al., 2014). The increased number of mitochondria with a short mitochondrial length and enhanced production of mitophagosomes in exercised RIHD mice accompanied by improved mitochondrial function further support the notion that exercise can activate mitochondrial fission and mitophagy to clear damaged organelles and promote the recovery of mitochondrial function. Consistently, the expression of a mitochondrial fission marker (DRP1) and mitophagy markers (PINK1 and LC3B) was significantly elevated in RIHD mice following aerobic exercise, but mitochondrial biogenesis and fusion marker expression remained non-significantly changed. Thus, we can speculate that exercise can promote the selective degradation of damaged mitochondria to restore metabolic homeostasis in cardiomyocytes.

In parallel with the enhanced fission and mitophagy, the elevated FNDC5/irisin expression induced by aerobic exercise can support the potential role of FNDC5/irisin in regulating mitochondrial turnover. While the molecular mechanism by which exercise regulated RIHD remains unknown, the positive correlation between serum irisin concentrations and the expression of mitochondrial fission and mitophagy markers may imply that irisin can regulate mitochondrial turnover through autocrine activity. Considering that FNDC5 is characterized with its fibronectin type III domain containing the Arg-Gly-Asp (RGD) sequence which accounts for the cell adhesive property and is similar to other RGD receptors (Main et al., 1992), FNDC5 could act as a transmembrane receptor despite its role as the precursor of irisin (Teufel et al., 2002; Erickson, 2013). Recent data also supported the role of FNDC5/irisin in alleviating oxidative stress caused by doxorubicin-induced cardiac toxicity in an AKT/mTOR-dependent pathway, with improved the sensitivity of the tumor response to chemotherapy (Liu et al., 2019; Zhang et al., 2019). Although we were not able to confirm the interaction between irisin and mitochondrial turnover markers at the protein level, our data suggest that FNDC5 and irisin are involved in the preservation of mitochondria by aerobic exercise, which may promote the elimination of accumulated dysfunctional mitochondria following RT.

As aerobic fitness reflects whole-body health, the beneficial effect of FNDC5/irisin on exercise fitness reported in the current study may be attributed to not only the recovery of cardiac function, but also the regulation of skeletal muscle metabolism through the autocrine function of irisin (Gomarasca et al., 2020). Therefore, FNDC5/irisin can act as an "exercise medicine" for cancer survivors undergoing thoracic RT who are not able to participate in an aerobic exercise rehabilitation program because of barriers experienced by cancer survivors, such as pain, fatigue, and environmental issues (Blaney et al., 2013; Romero et al., 2018).

There are several limitations of this study. First, we did not demonstrate the interaction between FNDC5/irisin and mitophagy markers at the protein level. However, the relationship of FNDC5/irisin and mitochondrial turnover revealed in this study might inspire further investigation regarding the new function of FNDC5/irisin in regulating MQC. Second, we were not able to assess the dynamic process of mitochondrial turnover by electron microscopy. Nevertheless, the shorter mitochondrial length accompanied by an increased number of mitophagosomes and alterations in mitochondrial turnover markers further support enhanced fission and mitophagy.

CONCLUSION

In conclusion, aerobic exercise may enhance the recovery of cardiac function and aerobic fitness by promoting mitochondrial fission and mitophagy through an FNDC5/irisin-dependent pathway. We have proven the efficacy of aerobic exercise in strengthening cardiovascular health in RIHD and revealed the potential of targeting FNDC5/irisin as an early intervention to prevent the development of RIHD.

DATA AVAILABILITY STATEMENT

The data that support the findings of the study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The animal study was reviewed and approved by The Animal Care and Use Committee of Chongqing Medical University.

AUTHOR CONTRIBUTIONS

WH and YT contributed equally to the conceptualization, study design, experiment conduction (including the establishment of animal model, western blotting, RT-PCR, histological analysis, and the assessment of mitochondrial function), data analysis, and drafting of the article. CL was responsible for conducting the exercise training of animal data analysis. ZY contributed to the conceptualization, supervision, project administration, and critical revision of the article. XZ, SH, and BT contributed

to the establishment of the animal model. All authors agreed on the final content of the article.

FUNDING

This study was supported by the National Natural Science Foundation of China (Grant No. 82100253 and 82002448), Chongqing Education Committee science and technology research project (KJQN202100463) and Chongqing Natural Science Foundation (Grant No. cstc2021jcyj-msxmX0268).

ACKNOWLEDGMENTS

The authors would like to thank American Journal Experts (AJE) for providing language help.

REFERENCES

Bhattacharya, S., and Asaithamby, A. (2016). Ionizing radiation and heart risks. *Semin. Cell. Dev. Biol.* 58, 14–25. doi: 10.1016/j.semcdb.2016.01.045

Blaney, J. M., Lowe-Strong, A., Rankin-Watt, J., Campbell, A., and Gracey, J. H. (2013). Cancer survivors' exercise barriers, facilitators and preferences in the context of fatigue, quality of life and physical activity participation: a questionnaire-survey. *Psycho-Oncology* 22, 186–194. doi: 10.1002/pon.2072

Bradley, J. D., Hu, C., Komaki, R. R., Masters, G. A., Blumenschein, G. R., and Schild, S. E. (2020). Long-term results of NRG oncology RTOG 0617: standard-versus high-dose chemoradiotherapy with or without cetuximab for unresectable stage III non-small-cell lung cancer. *J. Clin. Oncol.* 38, 706–714. doi: 10.1200/JCO.19.01162

Bradley, J. D., Paulus, R., Komaki, R., Masters, G., Blumenschein, G., and Schild, S. (2015). Standard-dose versus high-dose conformal radiotherapy with concurrent and consolidation carboplatin plus paclitaxel with or without cetuximab for patients with stage IIIA or IIIB non-small-cell lung cancer (RTOG 0617): a randomised, two-by-two factorial phase 3 study. *Lancet Oncol.* 16, 187–199. doi: 10.1016/S1470-2045(14)71207-0

Codella, R., Lanzoni, G., Zoso, A., Caumo, A., Montesano, A., Terruzzi, I. M., et al. (2015). Moderate intensity training impact on the inflammatory status and glycemic profiles in nod mice. *J. Diabetes Res.* 2015:737586. doi: 10.1155/2015/737586

Cullen, C. L., Wadowska, D. W., Singh, A., and Melekhovets, Y. (2010). Ultrastructural findings in feline corneal sequestra. *Vet. Ophthalmol.* 8, 295–303. doi: 10.1111/j.1463-5224.2005.00402.x

Erickson, H. P. (2013). Irisin and FNDC5 in retrospect: An exercise hormone or a transmembrane receptor? *Adipocytes* 2, 289–293. doi: 10.4161/adip.26082

Fernando, P., Bonen, A., and Hoffman-Goetz, L. (1993). Predicting submaximal oxygen consumption during treadmill running in mice. *Can. J. Physiol. Pharmacol.* 71, 854–857. doi: 10.1139/y93-128

Gomarasca, M., Banfi, G., and Lombardi, G. (2020). Myokines: The endocrine coupling of skeletal muscle and bone. *Adv. Clin. Chem.* 94, 155–218. doi: 10.1016/bs.acc.2019.07.010

Guo, S., Huang, Y., Zhang, Y., Huang, H., and Liu, T. (2019). Impacts of exercise intervention on different diseases and organ functions in mice. *J. Sport Health Sci.* 9, 53–73. doi: 10.1016/j.jshs.2019.07.004

Hargitai, R., Roivainen, P., Kis, D., Luukkonen, J., Sáfrány, G., and Seppälä, J. (2020). Mitochondrial DNA damage in the hair bulb: can it be used as a noninvasive biomarker of local exposure to low LET ionizing radiation? *Int. J. Radiat. Biol.* 96, 491–501. doi: 10.1080/09553002.2020.1704910

Iorga, A., Cunningham, C. M., Moazeni, S., Ruffenach, G., Umar, S., and Eghbali, M. (2017). The protective role of estrogen and estrogen receptors in cardiovascular disease and the controversial use of estrogen therapy. *Biol. Sex Differ.* 8:33. doi: 10.1186/s13293-017-0152-8

Kruse, J., Strootman, E. G., and Wondergem, J. (2003). Effects of amifostine on radiation-induced cardiac damage. *Acta Radiol. Ther. Phys. Biol.* 42, 4–9. doi: 10.1080/0891060310002168

Liu, J., Huang, Y., Liu, Y., and Chen, Y. (2019). Irisin enhances doxorubicin-induced cell apoptosis in pancreatic cancer by inhibiting the pi3k/akt/nf-κb pathway. *Med. Sci. Monit.* 25, 6085–6096. doi: 10.12659/MSM.917625

Ma, C., Ding, H., Deng, Y., Liu, H., and Yang, Y. (2021). Irisin: a new code uncover the relationship of skeletal muscle and cardiovascular health during exercise. *Front. Physiol.* 12:620608. doi: 10.3389/fphys.2021.620608

Main, A. L., Harvey, T. S., Baron, M., Boyd, J., and Campbell, I. D. (1992). The three-dimensional structure of the tenth type iii module of fibronectin: an insight into rgd-mediated interactions. *Cell* 71, 671–678. doi: 10.1016/0092-8674(92)90600-H

Martinet, W., Knaapen, M. W., De Meyer, G. R., Herman, A. G., and Kockx, M. M. (2002). Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. *Circulation* 106, 927–932. doi: 10.1161/01.CIR.0000026393.47805.21

McGale, P., Darby, S. C., Hall, P., Adolfsson, J., Bengtsson, N. O., and Bennet, A. M. (2011). Incidence of heart disease in 35,000 women treated with radiotherapy for breast cancer in Denmark and Sweden. *Radiother. Oncol.* 100, 167–175. doi: 10.1016/j.radonc.2011.06.016

Nazem, S., Rabiee, F., Ghaedi, K., Babashah, S., Sadeghizadeh, M., and Nasr-Esfahani, M. H. (2017). Fndc5 knockdown induced suppression of mitochondrial integrity and significantly decreased cardiac differentiation of mouse embryonic stem cells. *J. Cell. Biochem.* 119, 4528–4539. doi: 10.1002/jcb.26590

Ni, H. M., Williams, J. A., and Ding, W. X. (2015). Mitochondrial dynamics and mitochondrial quality control. *Redox Biol.* 4, 6–13. doi: 10.1016/j.redox.2014.11.006

Ouyang, C., Ye, Z., Cao, Z., Manaenko, A., and Sun, X. (2016). Methane attenuates myocardial ischemia injury in rats through anti-oxidative, anti-apoptotic and anti-inflammatory actions. *Free Radic. Biol. Med.* 90, 1–11. doi: 10.1016/j.freeradbiomed.2015.11.017

Piquereau, J., Caffin, F., Novotova, M., Lemaire, C., Veksler, V., and Garnier, A. (2013). Mitochondrial dynamics in the adult cardiomyocytes: which roles for a highly specialized cell? *Front. Physiol.* 4:102. doi: 10.3389/fphys.2013.00102

Puente, B. N., Kimura, W., Muralidhar, S. A., Moon, J., Amatruda, J. F., and Phelps, K. L. (2014). The oxygen-rich postnatal environment induces cardiomyocyte cell-cycle arrest through DNA damage response. *Cell* 157, 565–579. doi: 10.1016/j.cell.2014.03.032

Romero, S., Brown, J. C., Bauml, J. M., Hay, J. L., and Mao, J. J. (2018). Barriers to physical activity: a study of academic and community cancer survivors with pain. *J. Cancer Surviv.* 12, 744–752. doi: 10.1007/s11764-018-0711-y

Schofield, J. H., and Schafer, Z. T. (2021). Mitochondrial reactive oxygen species and mitophagy: a complex and nuanced relationship. *Antioxid. Redox Signal.* 34, 517–530. doi: 10.1089/ars.2020.8058

Scott, J. M., Nilsen, T. S., Gupta, D., and Jones, L. W. (2018a). Exercise therapy and cardiovascular toxicity in cancer. *Circulation* 137, 1176–1191. doi: 10.1161/CIRCULATIONAHA.117.024671

Scott, J. M., Zabor, E. C., Schwitzer, E., Koelwyn, G. J., Adams, S. C., and Nilsen, T. S. (2018b). Efficacy of exercise therapy on cardiorespiratory fitness in patients with cancer: a systematic review and meta-analysis. *J. Clin. Oncol.* 36:2297. doi: 10.1200/JCO.2017.77.5809

Seol, M. A., Jung, U., Eom, H. S., Kim, S. H., Park, H. R., and Jo, S. K. (2012). Prolonged expression of senescence markers in mice exposed to gamma-irradiation. *J. Vet. Sci.* 13, 331–338. doi: 10.4142/jvs.2012.13.4.331

Stenehjem, J. S., Smeland, K. B., Murbraech, K., Holte, H., Kvaløy, S., Thorsen, L., et al. (2016). Cardiorespiratory fitness in long-term lymphoma survivors after high-dose chemotherapy with autologous stem cell transplantation. *Br. J. Cancer* 115, 178–187. doi: 10.1038/bjc.2016.180

Sweegers, M. G., Altenburg, T. M., Brug, J., May, A. M., Van Vulpen, J. K., and Aaronson, N. K. (2019). Effects and moderators of exercise on muscle

strength, muscle function and aerobic fitness in patients with cancer: a meta-analysis of individual patient data. *Br. J. Sports Med.* 53, 812–812. doi: 10.1136/bjsports-2018-099191

Teufel, A., Malik, N., Mukhopadhyay, M., and Westphal, H. (2002). Frcp1 and Frcp2, two novel fibronectin type III repeat containing genes. *Gene* 297, 79–83. doi: 10.1016/S0378-1119(02)00828-4

Wuyang, H., Peng, W., Qingwei, C., and Chunqiu, L. (2020). Exercise enhances mitochondrial fission and mitophagy to improve myopathy following critical limb ischemia in elderly mice via the pgc1a/fndc5/irisin pathway. *Skelet. Muscle* 10:25. doi: 10.1186/s13395-020-00245-2

Yahyapour, R., Amini, P., Rezapoor, S., Rezaeyan, A., and Najafi, M. (2017). Targeting of inflammation for radiation protection and mitigation. *Curr. Mol. Pharmacol.* 11, 203–210. doi: 10.2174/1874467210666171108165641

Zeng, Y. C. (2016). Sestrin2 protects the myocardium against radiation-induced damage. *Radiat. Environ. Biophys.* 55, 195–202. doi: 10.1007/s00411-016-0643-8

Zhang, X., Hu, C., Kong, C. Y., Song, P., and Tang, Q. Z. (2019). Fndc5 alleviates oxidative stress and cardiomyocyte apoptosis in doxorubicin-induced cardiotoxicity via activating AKT. *Cell Death Differ.* 27, 540–555. doi: 10.1038/s41418-019-0372-z

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frontiers in Physiology

SYSTEMATIC REVIEW
published: 06 December 2021
doi: 10.3389/fphys.2021.788411

Ectodysplasin A/Ectodysplasin A Receptor System and Their Roles in Multiple Diseases

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Ectodysplasin A (EDA) is a member of the tumor necrosis factor (TNF) family of ligands that was initially reported to induce the formation of various ectodermal derivatives during normal prenatal development. EDA exerts its biological activity as two splice variants, namely, EDA-A1 and EDA-A2. The former binds to the EDA receptor (EDAR), resulting in the recruitment of the intracellular EDAR-associated death domain (EDARADD) adapter protein and the activation of the NF-κB signaling pathway, while the latter binds to a different receptor, EDA2R, also known as X-linked ectodermal dysplasia receptor (XEDAR). Inactivation mutation of the EDA gene or the genes coding for its receptors can result in hypohidrosis ectodermal dysplasia (HED), a condition that is characterized by oligotrichosis, edentulosis or oligodontia, and oligohidrosis or anhidrosis. Recently, as a new liver factor, EDA is gradually known and endowed with some new functions. EDA levels were observed to be upregulated in several metabolic diseases, such as non-alcoholic fatty liver disease (NAFLD), obesity, and insulin resistance. In addition, EDA and its receptors have been implicated in tumor pathogenesis through the regulation of tumor cell proliferation, apoptosis, differentiation, and migration. Here, we first review the role of EDA and its two-receptor system in various signaling pathways and then discuss the physiological and pathological roles of EDA and its receptors.

Keywords: ectodysplasin A, ectodysplasin A receptor, signaling pathways, metabolism, skeletal muscle homeostasis, tumorigenesis

OPEN ACCESS

Edited by:
Ana Cláudia Garcia De Oliveira Duarte, Federal University of São Carlos, Brazil

Reviewed by:
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Specialty section:
This article was submitted to Metabolic Physiology, a section of the journal Frontiers in Physiology

Received: *02 October 2021*
Accepted: *18 November 2021*
Published: *06 December 2021*

Citation:
Cai Z, Deng X, Jia J, Wang D and Yuan G (2021) Ectodysplasin A/Ectodysplasin A Receptor System and Their Roles in Multiple Diseases. Front. Physiol. 12:788411. doi: 10.3389/fphys.2021.788411

INTRODUCTION

The ectodysplasin A (EDA) gene is a member of the tumor necrosis factor (TNF) family located on the long arm of the X chromosome. The EDA transcript encodes the EDA protein that generates several splice variants, two of which—EDA-A1 (391 amino acids) and EDA-A2 (389 amino acids) which contain a TNF homology domain (Kere et al., 1996; Park et al., 2019). EDA-A1 is a homotrimer type II transmembrane protein consisting of a transmembrane domain, a furan protease recognition site, and a 19-repeat Gly-X-Y collagen domain (Liu et al., 2019). EDA-A1 binds to the ectodysplasin A receptor (EDAR), which contains 14 cysteine residues, of which only the 6 closest to the N-terminus approximate the canonical TNF receptor consensus (Headon and Overbeek, 1999). EDA-A1/EDAR binding results in the recruitment of the intracellular EDAR-associated death domain (EDARADD) adaptor protein (Kumar et al., 2001) and the activation of the NF-κB signaling pathway (Sadia, Foo et al., 2019). Mutations in any of EDA, EDAR, and

EDARADD can contribute to hypohidrotic ectodermal dysplasia (HED), which affects 1 in 10,000–100,000 newborns (Wohlfart et al., 2016; Feng et al., 2018; Liu et al., 2019). EDA-A2, which contains 2 amino acids less than EDA-A1, binds to a distinct receptor, EDA2R [also known as X-linked ectodermal dysplasia receptor (XEDAR)], indicating that the insertion of two amino acids into the ligand is a determinant of the specificity of receptor binding (Yan et al., 2000). Like EDA-A1, EDA-A2 also activates the NF-κB signaling pathway. TRAF6 can be recruited to the EDA-A2/EDA2R complex and thereby participate in the activation of the IκB kinase (IKK) complex, which is necessary for the translocation of NF-κB transcription factors into the nucleus (Yan et al., 2000). Studies have demonstrated that EDA-A2 is expressed in aging adipose, artery, heart, lung, muscle, and skin tissues and is associated with apoptosis (Yang et al., 2015; de Vries et al., 2017).

Although EDA and its receptors are known to be essential for ectodermal morphogenesis, their functions in disease pathology and related pathways are not well understood. EDA was recently identified as a liver-secreted protein involved in the occurrence and development of metabolic dysfunction. Loss- and gain-of-function studies have indicated that EDA, particularly the EDA-A2 isoform, regulates systemic glucose metabolism in type 2 diabetes mellitus (T2DM) (Awazawa et al., 2017). Additionally, the serum EDA-A2 level is dependent on T2DM, body mass index (BMI), and obesity (Yang et al., 2019). In patients with non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH), the plasma EDA content is high and is associated with deteriorating steatosis and fibrosis (Bayliss et al., 2021). In addition to the effects on HED and metabolic disorders, several studies have reported that EDA and its receptors are involved in cancer pathogenesis by regulating the apoptosis, proliferation, differentiation, and migration of cancer cells (Soraas and Stebbing, 2018; Vial et al., 2019; Li et al., 2020; Wang et al., 2020); however, this possibility remains controversial. Here, we review the role of EDA and its two-receptor system in various signaling pathways and then discuss the physiological and pathological roles associated with EDA and its receptors.

MULTIPLE SIGNALING PATHWAYS ACTIVATED BY ECTODYSPLASIN A

Recent studies have demonstrated that EDA and its receptors participate in multiple signaling pathways, including the Wnt/β-catenin (Wang et al., 2020), c-Jun N-terminal kinase (JNK) (Sinha et al., 2002), bone morphogenetic protein (BMP)/Smad (Han et al., 2018), and fibroblast growth factor (FGF) signaling pathways (Häärä et al., 2012; Huh et al., 2013).

Wang et al. (2020) reported that EDAR promoted tumor cell proliferation by inducing Wnt/β-catenin signaling. The expression levels of genes related to the Wnt/β-catenin signaling pathway were upregulated in EDA^{high} samples. Additionally, the loss of EDAR could interfere with β-catenin signaling/luciferase activity, while silencing EDAR in colorectal cancer (CRC) cells led to a decrease in β-catenin abundance compared with that in vector shRNA-treated cells. Combined, these observations support that EDAR can activate the Wnt/β-catenin signaling pathway. Similarly, Zhang et al. (2009) found that Wnt/β-catenin signaling was necessary for the activation of the EDA/EDAR/NF-κB signaling pathway in epithelial cells, as well as the subsequent morphological and molecular events required for hair follicle development.

The EDA/EDAR/EDARADD pathway-mediated regulation of target genes is known to be dependent on the activation of the NF-kB pathway (Sadier et al., 2014); however, Sinha et al. (2002) reported that EDA2R can also activate the NF-κB and JNK pathways in an EDA-A2-dependent manner. Transient transfection of cDNA encoding FLAG-labeled XEDAR-L or XEDAR-s subtypes resulted in similar activation of the NF-κB pathway, while significant activation of NF-κB signaling was also observed in EDA-A2-treated 293F-XEDAR cells (Sinha et al., 2002). A different study demonstrated that EDA-A2 may activate NF-κB pathway by TRAF6. TRAF6 may be recruited to ligated XEDAR and contribute to activation of the IKK complex for translocation of NF-κB transcription factors into the nucleus (Yan et al., 2000). In addition to NF-κB activation, EDA-A2/EDA2R can also activate the JNK pathway by stimulating a rapid and marked increase in JNK1 and JNK2 phosphorylation, thereby promoting the phosphorylation-induced activation of the c-Jun transcription factor.

BMP2, BMP4, and BMP7 are expressed in early dental epithelial cells and are key regulators of tooth morphogenesis (Zouvelou et al., 2009; Feng et al., 2011; Jia et al., 2016). A recent study showed that in dental epithelial cells, EDA-A1 significantly induced Nkx2-3 expression in the pharyngeal floor as well as in oral cavity and branchial arch ectoderm (Biben et al., 2002). Han et al. (2018) found that BMP signaling is involved in tooth tip formation and tooth germ development by regulating cell differentiation and proliferation in the enamel node. Furthermore, Nkx2-3 transfection inhibited cell proliferation and induced the expression of Bmp2 and Bmpr2 mRNA and the phosphorylation of Smad1/5/8 in dental epithelial stem cells (M3H1 cells). These results indicated that Nkx2-3 was induced by EDA-A1 as a target molecule of the EDA-A1/EDAR pathway in dental epithelial cells and subsequently regulated cell proliferation through the BMP signaling pathway. The effects of BMP on EDAR have also been investigated. Mou et al. (2006) identified an EDAR/BMP activation-inhibition mechanism in which EDA-A1 upregulates EDAR expression, which subsequently induces BMPs expression, leading, in turn, to the suppression of EDAR expression. EDAR inhibited BMP rapidly and correlated with the level of phospho-Smad1/5/8, which is the activated form of intracellular transducers of BMP signals.

Microarray analysis showed that FGF20 is one of the earliest EDA-induced genes in hair placodes and, thus, is a putative transcriptional target of EDA (Fliniaux et al., 2008; Lefebvre et al., 2012). Häärä et al. (2012) demonstrated that FGF20 removal in EDA overexpression mice resulted in the appearance of a third molar, similar to the phenotype observed with EDA loss-of-function, suggesting that FGF20 has a critical role in modulating the initiation and size of posterior molars. The authors further found that EDA could rapidly induce FGF20 expression and

FGF20 expression levels were related to EDA activity *in vivo*. Fgf20-null (Fgf20 $^{\beta Gal/\beta Gal}$) and Eda$^{-/-}$ mice share similar molar phenotypes, indicating that FGF20 might be a key mediator and a direct target of EDA signaling transduction. Huh et al. (2013) revealed that, compared with untreated controls, the FGF20 message was increased by 3.3- and 16-fold after 2 and 4 h of EDA treatment, respectively. The activation of EDA in the epidermis led to an increase in FGF20$^{\beta Gal}$ activity, whereas the opposite was observed with the loss of EDA/EDAR signaling. Further analysis also indicated that the absence of FGF20 led to the suppression of EDAR expression and, consequently, also EDAR signaling (**Figure 1**).

THE ROLE OF ECTODYSPLASIN A IN HAIR DEVELOPMENT

EDA is essential for the formation of skin appendages such as hair, teeth, sweat glands, and eyelids (Pinheiro and Freire-Maia, 1994). Recent studies have demonstrated that alopecia is independently associated with many concurrent conditions such as obesity, insulin resistance, and metabolic syndrome (Marks et al., 2019). HFD treatment can lead to hair loss in both young and aging mice, and multiple inductions of the hair cycle result in more severe and irreversible hair loss. Furthermore, HFD can accelerate both the anagen and telogen phases, shorten the hair growth cycle, and induce lipid droplet accumulation, ROS generation, and NF-κB signaling pathway activation in hair follicle stem cells (Morinaga et al., 2021). Liu et al. (2018) revealed that EDA mRNA and protein expression was higher in ear skin than in back skin. Moreover, alpaca ear hair is thick and straight with a long growth cycle, but its back hair is long, curved, and has a short growth cycle (Liu et al., 2011); these differences in growth cycle duration between the alpaca ear and back hair may be related to differential EDA expression. Kwack et al. (2019) explored the role of EDA-A2 and its receptor in hair follicles of mice and cultured human hair follicles. Compared with controls, the expression of the anti-apoptotic protein Bcl-2 was found to be decreased after rhEDA-A2 treatment, whereas that of the pro-apoptotic Bax and cleaved caspase-3 proteins was

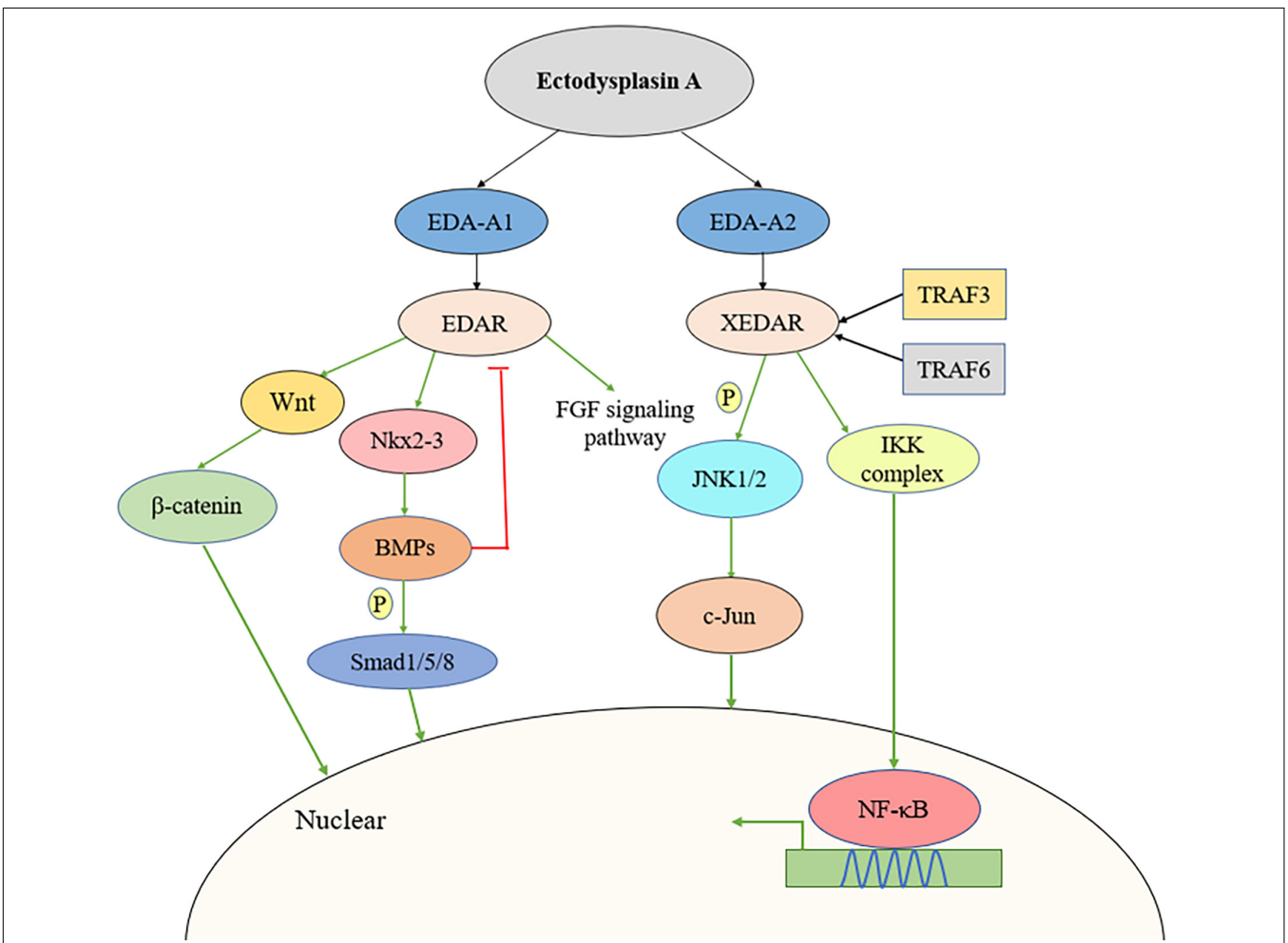

FIGURE 1 | Multiple signaling pathways activated by EDA including Wnt/β-catenin signaling pathway, JNK pathway, BMP/Smad pathway, and FGF pathway. BMP, bone morphogenetic protein; FGF, fibroblast growth factor; JNK, c-Jun N-terminal kinase.

significantly increased. EDA2R mRNA expression peaked in the late anagen phase. Consistent with this, skin hair follicles injected with EDA-A2 showed catagen, compared with the anagen in skin injected with heat-inactivated EDA-A2. Similarly, Jiang et al. (2012) demonstrated that EDA mRNA expression in hair follicles of goat skin tissue was higher during the catagen stage than during the telogen and anagen phases. These results indicate that EDA can induce the apoptosis of hair follicle cells and regulate the hair follicle growth cycle. However, the relationship among obesity, EDA, and alopecia is not clear. Whether a HFD can lead to alopecia by affecting the expression of EDA need to be further explored, as do the associated regulatory mechanisms.

THE ROLE OF ECTODYSPLASIN A IN SKELETAL MUSCLE HOMEOSTASIS

Skeletal muscle is extremely important in regulating whole-body energy expenditure and determining resting energy expenditure. It is the main site for glucose metabolism, fatty acid oxidation, and insulin activity and an organ with high adaptability to environmental pressures, such as obesity (Zurlo et al., 1990; Stewart and Rittweger, 2006). The skeletal muscle is heterogeneous and consists of different fiber phenotypes of varying oxidative and glycolytic properties (Simcocks et al., 2020). Targeting skeletal muscle is a possible therapeutic strategy for improving metabolic homeostasis (Rossi et al., 2016). Newton et al. (2004) found that EDA-A2 might play a role in skeletal muscle homeostasis in an EDA2R expression-dependent manner. Myosin light-chain 2 (MLCH). EDA-A2 transgenic mice showed skeletal muscle degeneration in both weight-bearing and non-weight-bearing muscles. Importantly, EDA2R deficiency alleviated myodegeneration due to EDA-A2 overexpression. Additionally, a comparative analysis of EDA2R expression between skeletal muscle from wild-type and MLC2. EDA-A2 transgenic mice using an EDA2R riboprobe revealed that the EDA2R signal was strongest at sites of muscle damage in EDA-A2 transgenic mice. Recombinant human EDA-A2 can promote IκBα phosphorylation in normal human skeletal muscle cells, suggesting that EDA-A2 might cause muscle degeneration through IκBα phosphorylation; however, how EDA-A2 might exert these myodegenerative effects remains unknown. Studies have also demonstrated that treating 293E cells with recombinant human EDA-A2 can result in the activation of the IKK complex, leading to the phosphorylation of IκBα (Yan et al., 2000). In conclusion, EDA-A2 might instigate skeletal muscle degeneration by promoting IκBα phosphorylation through EDA2R. IκBα might be the downstream mechanism of EDA-A2, but whether it is involved in other diseases and deeper mechanisms need to be further explored.

THE ROLE OF ECTODYSPLASIN A IN METABOLIC DISEASES

Glucose and lipid metabolism are closely linked and the stability of these two metabolic pathways is critical for maintaining body organ function (Du et al., 2021). Insulin resistance is highly associated with the occurrence and development of glucose metabolism disorder (James et al., 2021). Insulin resistance is defined as a reduction in the metabolic response of insulin-responsive cells to insulin or an impaired/reduced response of blood glucose levels to circulating insulin at the systemic level (Czech, 2017). In the liver, insulin not only regulates glucose production and utilization, but also has a broader influence on lipid metabolism (Chen, 2021). When circulating blood glucose levels are elevated, pancreatic β cells secrete insulin, which binds to hepatic insulin receptor (INSR). The receptor undergoes autophosphorylation, leading to the recruitment and phosphorylation of insulin receptor substrates (IRSs) that, in turn, activate downstream genes, finally resulting in AKT phosphorylation and activation. Once fully activated, AKT is involved in many downstream pathways, through which it regulates a variety of metabolic processes, including gluconeogenesis, glycolysis, glycogen synthesis, and lipid synthesis (Cherrington et al., 2007; Czech, 2017; Edgerton et al., 2017; Scherer, 2019). Insulin plays two main roles in the liver, namely, inhibiting glucose production (gluconeogenesis) and activating fatty acid and triglyceride (TG) synthesis (lipogenesis). Under the insulin resistance state, insulin does not inhibit gluconeogenesis; instead, it paradoxically over-activates adipogenesis, which leads to a fatal combination of hyperglycemia and hypertriglyceridemia (Staehr et al., 2004).

Insulin resistance in the liver is often accompanied by dyslipidemia and the occurrence of NAFLD (Byrne and Targher, 2015). The increase of fatty acid level is the main cause of hepatic steatosis and insulin resistance. Glucose feeding (and increased insulin levels) increases the production of new fat and stimulates the expression, nuclear localization, and transcriptional activity of sterol regulatory element-binding transcription factor 1 (SREBP1c) and other transcription factors (Horton et al., 2002). After activation of SREBP1c, the expression of genes related to *de novo* lipid synthesis, such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), is upregulated, thereby increasing the production of fatty acids (Horton et al., 2002; Alves-Bezerra and Cohen, 2017). Peroxisome proliferator-activated receptor gamma (PPARγ), another nuclear hormone receptor, contributes to energy storage primarily by promoting adipogenesis and lipid synthesis and displays the highest expression levels in white adipose tissue (WAT); meanwhile, the complete transcriptional activity of PPARs requires the binding of homologous lipid ligands and heterodimerization with retinoid-X receptor (RXR), also a nuclear receptor. PPARγ phosphorylation may restore insulin sensitivity by enhancing PPARγ function. In contrast, PPARγ dominant-negative mutations result in hypertension and insulin resistance, suggesting that a relationship exists between PPARγ function and metabolic syndrome (Plutzky, 2011; Christofides et al., 2021).

Several studies have shown that EDA, a recently identified hepatokine, is mainly expressed in the liver and can be secreted into the circulatory system to participate in energy and glycolipid metabolism (Awazawa et al., 2017; Yang et al., 2019; Bayliss et al., 2021). Awazawa et al. (2017) found that the expression

levels of EDA, corresponding to miR-676, were higher in the livers of *db/db* mice than in those of control mice. Clinical studies have shown that in humans, EDA expression in the liver is positively correlated with liver fat content, visceral fat area, and NASH scores. Additionally, EDA expression was significantly decreased after surgery, and was accompanied by weight loss and improved insulin sensitivity. A case-control study (Yang et al., 2019) showed that the serum EDA-A2 concentration in patients with NAFLD was higher than that in the controls. The frequency of NAFLD increased with increasing EDA-A2 levels. ROC curve analysis also revealed that EDA-A2 levels could predict the presence of NAFLD. Significant and positive associations were found between EDA-A2 levels and BMI, waist-to-hip ratio (WHR), fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), and homeostasis model assessment of insulin resistance (HOMA-IR), a series of anthropometric parameters and some parameters of glucose metabolism and insulin function. In addition, Bayliss et al. (2021) found that liver EDA mRNA levels were higher in NASH group than in those without NAFLD; however, no difference was detected between NAFL and non-NAFLD patients. Compared with patients without NAFLD, plasma EDA concentrations were increased in both the NAFL and NASH groups and were positively correlated with the degree of steatosis. Interestingly, the authors proposed that plasma EDA was not a reliable biomarker for NAFLD and could not discriminate between NAFL and NASH. Several reasons can explain the differences between these two studies. First, Yang et al. assessed serum EDA-A2, while Bayliss et al. assessed total serum EDA. EDA-A1 and EDA-A2 share a TNF homologous domain and differ by only two amino acids. Moreover, the proportion of EDA-A2 in total EDA is unclear. The second main reason relates to the different criteria used for NAFLD diagnosis. Yang et al. used ultrasonography and graded NAFLD based on the Chinese Standard, while in Bayliss's study, NAFLD was determined using liver biopsy and histological assessment. In conclusion, it remains unknown whether EDA concentrations can predict the presence of NAFLD, and additional studies are required to address the above-described discrepancies.

In experiments *in vivo*, evaluation based on enzyme-linked immunosorbent assay (ELISA) showed that the plasma EDA concentrations were higher in *db/db* mice and obese mice than in their respective control groups (Awazawa et al., 2017). EDA-A2 overexpression resulted in higher glucose concentration in the glucose tolerance test and lower energy consumption relative to control (GFP-AAV-injected) mice. Immunoblot analyses showed a comparable upregulation of insulin receptor substrate 1 (IRS1) phosphorylation at Ser307 in skeletal muscle. Similarly, suppressing EDA expression decreased blood glucose concentrations in an insulin tolerance test but did not influence weight, energy expenditure, exercise ability, or food intake. Furthermore, a different study (Yang et al., 2019) showed that EDA knockdown attenuated hepatic lipogenesis in HepG2 cells. The TG content in free fatty acid (FFA) + EDA small interfering RNA (siRNA)-treated cells was significantly lower when compared with that in cells treated with FFAs alone. The effect of EDA on liver lipid metabolism might be exerted through the regulation of lipolysis- and lipogenesis-related genes such as SREBP1c and the key fatty acid synthesis-associated enzymes FAS and ACC. The TG content of mice fed a high-fat diet (HFD) for 8 weeks was significantly increased, while the HFD-induced increase in the numbers of lipid droplets was markedly weakened in EDA-depleted mice. In addition, EDA knockdown inhibited serum aspartate transaminase (AST) and alanine transaminase (ALT) activity, but not that of alkaline phosphatase (ALP).

The mechanism involved in how obesity leads to the upregulation of EDA expression in the liver has also been investigated. Awazawa et al. (2017) found that the combined expression of PPARγ and RXR-α induced EDA promoter activity and EDA mRNA expression in Hepa1-6 cells, while the overexpression of either factor alone or the pharmacological activation of PPARγ failed to induce EDA expression in the liver. JNK is one of the best-characterized signal transducers in obesity and insulin resistance (Pal et al., 2016; Solinas and Becattini, 2017). *In vivo* administration of EDA-A2 increased JNK phosphorylation, while JNK phosphorylation levels were found to be higher in mice injected with EDA-A2-AAV than in control mice (Awazawa et al., 2017).

Collectively, these findings suggest that EDA secreted from liver tissues is associated with insulin resistance, diabetes, and NAFLD and that stimulating EDA or blocking EDA signaling may modulate hepatic steatosis and insulin resistance; however, the specific underlying mechanisms remain elusive (**Figure 2**). In addition, although some studies have shown that EDA-A2 has a role in metabolic diseases, other studies have been unable to distinguish whether this regulation is exerted by EDA-A1 or EDA-A2. This highlights the need to investigate the impact of EDA-A1 and EDA-A2 on metabolic disorders separately, including the detection of their concentrations in circulating blood and their expression levels in liver tissues, as well as their specific effects using loss- and gain-of-function methods.

THE ROLE OF ECTODYSPLASIN A IN DIABETIC NEPHROPATHY

Diabetic nephropathy (DN) is a common complication in patients with diabetes. It is the main cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD). DN is characterized by podocyte apoptosis, mesangial cell proliferation, matrix expansion, and glomerular and tubulointerstitial fibrosis (Wang et al., 2021). Studies have indicated that EDA may have a role in the development of DN. The expression of EDA2R has been reported to be upregulated in diabetic kidneys (in both type 1 and type 2 diabetes) (Watanabe et al., 2013; Brennan et al., 2018). Recently, one study also showed that the mRNA and protein expression of EDA2R was increased in both type 1 (STZ injection) and type 2 (*Btbr* $^{ob/ob}$) diabetic mice relative to controls. Consistent with the results in animals, EDA2R was also found to be highly expressed in podocytes treated with high glucose concentrations *in vitro* as well as in glomerular podocytes of diabetic patients. Moreover, EDA2R might provoke podocyte injury through the generation of reactive oxygen species (ROS). EDA2R overexpression was shown to inhibit the expression of anti-apoptotic molecules such as Mcl-1 and Bcl-2 while enhancing that of pro-apoptotic molecules

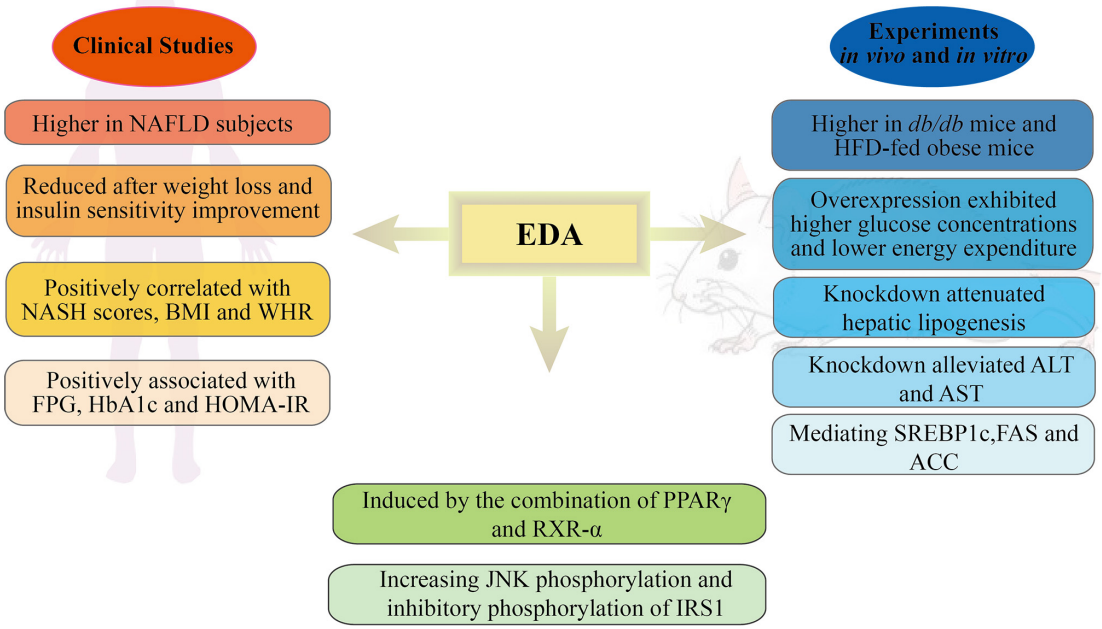

FIGURE 2 | Schematic representation of the roles and mechanisms of EDA in metabolic disorders. NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; BMI, body mass index; WHR, waist-to-hip ratio; FPG, fasting plasma glucose; HbA1c, hemoglobin A 1c; HOMA-IR, homeostasis model assessment of insulin resistance; HFG, high-fat diet; ALT, alanine transaminase; AST, aspartate transaminase; SREBP1c, sterol regulatory element binding transcription factor 1; FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; PPARγ, proliferator-activated receptor γ; RXR-α, retinoid-X receptor-α; IRS1, insulin receptor substrate 1.

such as Bax and cleaved caspase-3. EDA2R increased ROS production in podocytes, while inhibiting ROS generation could weaken EDA2R-mediated podocyte injury. In addition, EDA2R-knockdown podocytes displayed attenuated ROS production under stimulation with high glucose concentrations. The silencing of EDA2R expression partially relieved the occurrence of high glucose-induced apoptosis and dedifferentiation (Lan et al., 2020). EDA2R might exacerbate the development of DN by regulating the apoptosis and dedifferentiation of podocytes and enhancing the generation of ROS. It is not clear whether similar mechanisms mediate the involvement of EDA2R in glycolipid metabolism and DN or whether EDA2R can serve as a novel therapeutic target for the treatment of patients with DN.

THE ROLE OF ECTODYSPLASIN A IN TUMORIGENESIS

Although EDA has been proposed to play a regulatory role in cell proliferation and differentiation, this possibility remains controversial and warrants further investigation. For instance, the epidermal growth factor receptor (EGFR) gene is one of the most frequently mutated genes in lung cancer, particularly in non-small cell lung adenocarcinoma (Rosell et al., 2009). Soraas and Stebbing (2018) identified a significant and positive correlation between EDAR polymorphism and EGFR mutation frequencies, indicating that the EDAR gene might be associated with lung cancer and may have potential as a biomarker for the diagnosis of this disease. In a different study (Wang et al., 2020), the authors showed that the mRNA and protein expression of EDAR was upregulated in CRC tissues and CRC cell lines relative to their respective controls. Additionally, CRC patients with high EDAR expression have poor clinical outcomes, whereas those with low EDAR expression showed improved overall survival rates. Compared with vector controls, shRNA-mediated knockdown of EDAR significantly reduced the size and number of CRC cell colonies and induced cell cycle arrest in the G1 phase. *In vivo*, the tumor burden of mice transplanted with shEDAR-transduced tumor cells was significantly alleviated, and the tumor volume of EDAR-deficient mice was less than 1,000 mm^3. In addition, Li et al. (2020) showed that EDARADD was highly expressed in head and neck squamous cell carcinoma (HNSCC) tissues while EDARADD expression was associated with the degree of tumor differentiation and local recurrence in tongue squamous cell carcinoma (TSCC). Furthermore, EDARADD knockdown in TSCC cells affected clonogenicity, induced apoptosis, suppressed proliferation, and reduced the expression of NF-κBp65, MYC, and Bcl-2. NF-κB plays a broad role in cell proliferation and mediates apoptosis, especially its RelA (p65) subunit (Giridharan and Srinivasan, 2018; Zeng et al., 2019). MYC is located downstream of NF-κB and promotes cell growth and proliferation, while Bcl-2 family proteins regulate apoptosis (Adams and Cory, 1998; Zhao et al., 2018). These results all demonstrate that both EDAR and EDARADD are involved in cancer pathogenesis; in contrast, however, Vial et al. (2019) revealed that EDAR acts as a tumor suppressor in melanoma. The authors observed a marked decrease in EDAR expression in malignant melanoma compared with that in benign nevi. Each EDAR mutation (T167I, E254K, P409L, and V416M) significantly impaired EDAR

pro-apoptotic activity. EDAR knockout mice were reported to develop melanoma lesions over the first 400 days, a phenotype that was linked with reduced survival. Together, these findings suggest that EDA is closely linked with cancer cell proliferation, migration, differentiation, and local recurrence. Whether EDA exerts positive or negative effects is likely to be dependent on tumor type, and requires further investigation.

CONCLUSION

In summary, EDA, a hepatokine, may be associated with a variety of diseases, such as metabolic disorders, DN, and cancer, and may be a link among insulin resistance, T2DM, obesity, and NAFLD. Our review highlighted the potential roles of this hepatokine and the possibility of targeting the EDA signaling pathway in different pathophysiological processes. Importantly, elevated circulating EDA levels are closely associated with the incidence of NAFLD, rendering EDA a potential biomarker for the clinical diagnosis of this condition. However, further clinical investigation is warranted to confirm this possibility.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

ZC and GY conceived and designed the review. ZC and XD analyzed the data and wrote the original draft of the manuscript. DW and JJ revised the final manuscript. All authors read and approved the final manuscript.

FUNDING

This work was supported by the National Natural Science Foundation of China (81870548 and 81570721), the Social Development Project of Jiangsu Province (BE2018692), the Natural Science Foundation of Jiangsu Province, China (BK20191222 and BK20151331), the High Caliber Medical Personnel Foundation of Jiangsu Province (LGY2016053), the Six Talent Peaks Project in Jiangsu Province (2015-WSN-006), and the Fifth "169 project" Scientific Research Project of Zhenjiang City, Jiangsu Province.

ACKNOWLEDGMENTS

We thank all authors of this manuscript who have made substantial contributions to this work.

REFERENCES

Adams, J., and Cory, S. (1998). The Bcl-2 protein family: arbiters of cell survival. *Science* 281, 1322–1326. doi: 10.1126/science.281.5381.1322

Alves-Bezerra, M., and Cohen, D. (2017). Triglyceride metabolism in the liver. *Compr. Physiol.* 8, 1–8. doi: 10.1002/cphy.c170012

Awazawa, M., Gabel, P., Tsaousidou, E., Nolte, H., Krüger, M., Schmitz, J., et al. (2017). A microRNA screen reveals that elevated hepatic ectodysplasin A expression contributes to obesity-induced insulin resistance in skeletal muscle. *Nat. Med.* 23, 1466–1473. doi: 10.1038/nm.4420

Bayliss, J., Ooi, G., De Nardo, W., Shah, Y., Montgomery, M., McLean, C., et al. (2021). Ectodysplasin a is increased in non-alcoholic fatty liver disease, but is not associated with type 2 diabetes. *Front. Endocrinol.* 12:642432. doi: 10.3389/fendo.2021.642432

Biben, C., Wang, C., and Harvey, R. (2002). NK-2 class homeobox genes and pharyngeal/oral patterning: Nkx2-3 is required for salivary gland and tooth morphogenesis. *Int. J. Dev. Biol.* 46, 415–422.

Brennan, E., Mohan, M., McClelland, A., Tikellis, C., Ziemann, M., Kaspi, A., et al. (2018). Lipoxins regulate the early growth response-1 network and reverse diabetic kidney disease. *J. Am. Soc. Nephrol.* 29, 1437–1448. doi: 10.1681/asn.2017101112

Byrne, C., and Targher, G. (2015). NAFLD: a multisystem disease. *J. Hepatol.* 62, S47–S64. doi: 10.1016/j.jhep.2014.12.012

Chen, G. (2021). The interactions of insulin and Vitamin A signaling systems for the regulation of hepatic glucose and lipid metabolism. *Cells* 10:2160. doi: 10.3390/cells10082160

Cherrington, A., Moore, M., Sindelar, D., and Edgerton, D. (2007). Insulin action on the liver in vivo. *Biochem. Soc. Trans.* 35, 1171–1174. doi: 10.1042/bst0351171

Christofides, A., Konstantinidou, E., Jani, C., and Boussiotis, V. (2021). The role of peroxisome proliferator-activated receptors (PPAR) in immune responses. *Metab. Clin. Exp.* 114:154338. doi: 10.1016/j.metabol.2020.154338

Czech, M. (2017). Insulin action and resistance in obesity and type 2 diabetes. *Nat. Med.* 23, 804–814. doi: 10.1038/nm.4350

de Vries, M., Faiz, A., Woldhuis, R., Postma, D., de Jong, T., Sin, D., et al. (2017). Lung tissue gene-expression signature for the ageing lung in COPD. *Thorax* doi: 10.1136/thoraxjnl-2017-210074 [Epub Online ahead of print].

Du, H., Zhao, Y., Yin, Z., Wang, D., and Chen, C. (2021). The role of miR-320 in glucose and lipid metabolism disorder-associated diseases. *Int. J. Biol. Sci.* 17, 402–416. doi: 10.7150/ijbs.53419

Edgerton, D., Kraft, G., Smith, M., Farmer, B., Williams, P., Coate, K., et al. (2017). Insulin's direct hepatic effect explains the inhibition of glucose production caused by insulin secretion. *JCI insight.* 2:e91863. doi: 10.1172/jci.insight.91863

Feng, J., Yang, G., Yuan, G., Gluhak-Heinrich, J., Yang, W., Wang, L., et al. (2011). Abnormalities in the enamel in bmp2-deficient mice. *Cells Tissues Organs* 194, 216–221. doi: 10.1159/000324644

Feng, X., Weng, C., Wei, T., Sun, J., Huang, F., Yu, P., et al. (2018). Two EDA gene mutations in chinese patients with hypohidrotic ectodermal dysplasia. *J. Eur. Acad. Dermatol. Venereol.* 32, e324–e326. doi: 10.1111/jdv.14874

Fliniaux, I., Mikkola, M., Lefebvre, S., and Thesleff, I. (2008). Identification of dkk4 as a target of Eda-A1/Edar pathway reveals an unexpected role of ectodysplasin as inhibitor of Wnt signalling in ectodermal placodes. *Dev. Biol.* 320, 60–71. doi: 10.1016/j.ydbio.2008.04.023

Giridharan, S., and Srinivasan, M. (2018). Mechanisms of NF-κB p65 and strategies for therapeutic manipulation. *J. Inflamm. Res.* 11, 407–419. doi: 10.2147/jir.S140188

Häärä, O., Harjunmaa, E., Lindfors, P., Huh, S., Fliniaux, I., Åberg, T., et al. (2012). Ectodysplasin regulates activator-inhibitor balance in murine tooth development through Fgf20 signaling. *Development* 139, 3189–3199. doi: 10.1242/dev.079558

Han, X., Yoshizaki, K., Miyazaki, K., Arai, C., Funada, K., Yuta, T., et al. (2018). The transcription factor NKX2-3 mediates p21 expression and ectodysplasin-A signaling in the enamel knot for cusp formation in tooth development. *J. Biol. Chem.* 293, 14572–14584. doi: 10.1074/jbc.RA118.003373

Headon, D., and Overbeek, P. (1999). Involvement of a novel Tnf receptor homologue in hair follicle induction. *Nature genetics* 22, 370–374. doi: 10.1038/11943

Horton, J., Goldstein, J., and Brown, M. (2002). SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* 109, 1125–1131. doi: 10.1172/jci15593

Huh, S., Närhi, K., Lindfors, P., Häärä, O., Yang, L., Ornitz, D., et al. (2013). Fgf20 governs formation of primary and secondary dermal condensations in developing hair follicles. *Genes Dev.* 27, 450–458. doi: 10.1101/gad.198945.112

James, D., Stöckli, J., and Birnbaum, M. (2021). The aetiology and molecular landscape of insulin resistance. *Nat. Rev. Mol. Cell Biol.* 22, 751–771. doi: 10.1038/s41580-021-00390-6

Jia, S., Kwon, H., Lan, Y., Zhou, J., Liu, H., and Jiang, R. (2016). Bmp4-Msx1 signaling and Osr2 control tooth organogenesis through antagonistic regulation of secreted Wnt antagonists. *Dev. Biol.* 420, 110–119. doi: 10.1016/j.ydbio.2016.10.001

Jiang, W., Xue, P., He, Y., and Chen, Y. (2012). Molecular cloning, sequence analysis and expression of goat Eda gene. *J. Northwest A&F Univ.* 40, 7–12. doi: 10.1007/s11783-011-0280-z

Kere, J., Srivastava, A., Montonen, O., Zonana, J., Thomas, N., Ferguson, B., et al. (1996). X-linked anhidrotic (hypohidrotic) ectodermal dysplasia is caused by mutation in a novel transmembrane protein. *Nature genetics* 13, 409–416. doi: 10.1038/ng0895-409

Kumar, A., Eby, M., Sinha, S., Jasmin, A., and Chaudhary, P. (2001). The ectodermal dysplasia receptor activates the nuclear factor-kappaB, JNK, and cell death pathways and binds to ectodysplasin A. *J. Biol. Chem.* 276, 2668–2677. doi: 10.1074/jbc.M008356200

Kwack, M., Kim, J., and Kim, M. (2019). Ectodysplasin-A2 induces apoptosis in cultured human hair follicle cells and promotes regression of hair follicles in mice. *Biochem. Biophys. Res. Commun.* 520, 428–433. doi: 10.1016/j.bbrc.2019.10.031

Lan, X., Kumar, V., Jha, A., Aslam, R., Wang, H., Chen, K., et al. (2020). EDA2R mediates podocyte injury in high glucose milieu. *Biochimie* 174, 74–83. doi: 10.1016/j.biochi.2020.04.003

Lefebvre, S., Fliniaux, I., Schneider, P., and Mikkola, M. (2012). Identification of ectodysplasin target genes reveals the involvement of chemokines in hair development. *J. Invest. Dermatol.* 132, 1094–1102. doi: 10.1038/jid.2011.453

Li, M., Bai, Y., Han, K., Li, X., and Meng, J. (2020). Knockdown of ectodysplasin-A receptor-associated adaptor protein exerts a tumor-suppressive effect in tongue squamous cell carcinoma cells. *Exp. Ther. Med.* 19, 3337–3347. doi: 10.3892/etm.2020.8578

Liu, D., Zhang, Z., He, X., Dong, Y., Zhu, Z., Bai, R., et al. (2011). FGF5 Expression and Immunolocalization in Back and Ear Skin of Young Alpaca (*Lama pacos*). *Chin. J. Biochem. Mol. Biol.* 27, 473–479. doi: 10.13865/j.cnki.cjbmb.2011.05.009

Liu, G., Wang, X., Qin, M., Sun, L., and Zhu, J. (2019). A novel missense mutation p.S305R of EDA gene causes XLHED in a Chinese family. *Arch. Oral Biol.* 107:104507. doi: 10.1016/j.archoralbio.2019.104507

Liu, N., Niu, S., Cao, X., Cheng, J., Gao, S., Yu, X., et al. (2018). Let-7b regulates alpaca hair growth by downregulating ectodysplasin A. *Mol. Med. Rep.* 17, 4688–4694. doi: 10.3892/mmr.2018.8442

Marks, D., Penzi, L., Ibler, E., Manatis-Lornell, A., Hagigeorges, D., Yasuda, M., et al. (2019). The medical and psychosocial associations of alopecia: recognizing hair loss as more than a cosmetic concern. *Am. J. Clin. Dermatol.* 20, 195–200. doi: 10.1007/s40257-018-0405-2

Morinaga, H., Mohri, Y., Grachtchouk, M., Asakawa, K., Matsumura, H., Oshima, M., et al. (2021). Obesity accelerates hair thinning by stem cell-centric converging mechanisms. *Nature* 595, 266–271. doi: 10.1038/s41586-021-03624-x

Mou, C., Jackson, B., Schneider, P., Overbeek, P., and Headon, D. (2006). Generation of the primary hair follicle pattern. *Proc. Natl. Acad. Sci. U. S. A.* 103, 9075–9080. doi: 10.1073/pnas.0600825103

Newton, K., French, D., Yan, M., Frantz, G., and Dixit, V. (2004). Myodegeneration in EDA-A2 transgenic mice is prevented by XEDAR deficiency. *Mol. Cell. Biol.* 24, 1608–1613. doi: 10.1128/mcb.24.4.1608-1613.2004

Pal, M., Febbraio, M., and Lancaster, G. (2016). The roles of c-Jun NH2-terminal kinases (JNKs) in obesity and insulin resistance. *J. Physiol.* 594, 267–279. doi: 10.1113/jp271457

Park, J., Ko, J., and Chae, J. (2019). Novel and Private EDA mutations and clinical phenotypes of Korean patients with X-Linked hypohidrotic ectodermal dysplasia. *Cytogenet. Genome Res.* 158, 1–9. doi: 10.1159/000500214

Pinheiro, M., and Freire-Maia, N. (1994). Ectodermal dysplasias: a clinical classification and a causal review. *Am. J. Med. Genet.* 53, 153–162. doi: 10.1002/ajmg.1320530207

Plutzky, J. (2011). The PPAR-RXR transcriptional complex in the vasculature: energy in the balance. *Circ. Res.* 108, 1002–1016. doi: 10.1161/circresaha.110.226860

Rosell, R., Moran, T., Queralt, C., Porta, R., Cardenal, F., Camps, C., et al. (2009). Screening for epidermal growth factor receptor mutations in lung cancer. *New Engl. J. Med.* 361, 958–967. doi: 10.1056/NEJMoa0904554

Rossi, F., Bellini, G., Luongo, L., Manzo, I., Tolone, S., Tortora, C., et al. (2016). Cannabinoid Receptor 2 as Antiobesity Target: inflammation, fat storage, and browning modulation. *J. Clin. Endocrinol. Metab.* 101, 3469–3478. doi: 10.1210/jc.2015-4381

Sadia, Foo, J., Khor, C., Jelani, M., and Ali, G. (2019). A recurrent missense mutation in the EDAR gene causes severe autosomal recessive hypohidrotic ectodermal dysplasia in two consanguineous Kashmiri families. *J. Gene Med.* 21:e3113. doi: 10.1002/jgm.3113

Sadier, A., Viriot, L., Pantalacci, S., and Laudet, V. (2014). The ectodysplasin pathway: from diseases to adaptations. *Trends Genet.* 30, 24–31. doi: 10.1016/j.tig.2013.08.006

Scherer, P. (2019). The many secret lives of adipocytes: implications for diabetes. *Diabetologia* 62, 223–232. doi: 10.1007/s00125-018-4777-x

Simcocks, A., O'Keefe, L., Jenkin, K., Cornall, L., Grinfeld, E., Mathai, M., et al. (2020). The Role of Atypical Cannabinoid Ligands O-1602 and O-1918 on Skeletal Muscle Homeostasis with a Focus on Obesity. *Int. J. Mol. Sci.* 21:5922. doi: 10.3390/ijms21165922

Sinha, S., Zachariah, S., Quiñones, H., Shindo, M., and Chaudhary, P. (2002). Role of TRAF3 and −6 in the activation of the NF-kappa B and JNK pathways by X-linked ectodermal dysplasia receptor. *J. Biol. Chem.* 277, 44953–44961. doi: 10.1074/jbc.M207923200

Solinas, G., and Becattini, B. (2017). JNK at the crossroad of obesity, insulin resistance, and cell stress response. *Mol. Metab.* 6, 174–184. doi: 10.1016/j.molmet.2016.12.001

Soraas, L., and Stebbing, J. (2018). Geographic Variation in EGFR Mutation Frequency in Lung Adenocarcinoma May Be Explained by Interethnic Genetic Variation. *J. Thorac. Oncol.* 13, 454–458. doi: 10.1016/j.jtho.2017.11.128

Staehr, P., Hother-Nielsen, O., and Beck-Nielsen, H. (2004). The role of the liver in type 2 diabetes. *Rev. Endocr. Metab. Disord.* 5, 105–110. doi: 10.1023/B:REMD.0000021431.90494.0c

Stewart, C., and Rittweger, J. (2006). Adaptive processes in skeletal muscle: molecular regulators and genetic influences. *J. Musculoskeletal Neuro. Interact.* 6, 73–86.

Vial, J., Royet, A., Cassier, P., Tortereau, A., Dinvaut, S., Maillet, D., et al. (2019). The Ectodysplasin receptor EDAR acts as a tumor suppressor in melanoma by conditionally inducing cell death. *Cell Death Differ.* 26, 443–454. doi: 10.1038/s41418-018-0128-1

Wang, B., Liang, Y., Chai, X., Chen, S., Ye, Z., Li, R., et al. (2020). Ectodysplasin A receptor (EDAR) promotes colorectal cancer cell proliferation via regulation of the Wnt/β-catenin signaling pathway. *Exp. Cell Res.* 395:112170. doi: 10.1016/j.yexcr.2020.112170

Wang, L., Wang, H., Liu, T., and Lan, H. (2021). TGF-Beta as a Master Regulator of Diabetic Nephropathy. *Int. J. Mol. Sci.* 22:7881. doi: 10.3390/ijms22157881

Watanabe, T., Tomioka, N., Doshi, M., Watanabe, S., Tsuchiya, M., and Hosoyamada, M. (2013). Macrophage migration inhibitory factor is a possible candidate for the induction of microalbuminuria in diabetic db/db mice. *Biol. Pharma. Bull.* 36, 741–747. doi: 10.1248/bpb.b12-00741

Wohlfart, S., Söder, S., Smahi, A., and Schneider, H. (2016). A novel missense mutation in the gene EDARADD associated with an unusual phenotype of hypohidrotic ectodermal dysplasia. *Am. J. Med. Genet. Part A.* 170A, 249–253. doi: 10.1002/ajmg.a.37412

Yan, M., Wang, L., Hymowitz, S., Schilbach, S., Lee, J., Goddard, A., et al. (2000). Two-amino acid molecular switch in an epithelial morphogen that regulates binding to two distinct receptors. *Science* 290, 523–527. doi: 10.1126/science.290.5491.523

Yang, J., Huang, T., Petralia, F., Long, Q., Zhang, B., Argmann, C., et al. (2015). Synchronized age-related gene expression changes across multiple tissues in

human and the link to complex diseases. *Sci. Rep.* 5:15145. doi: 10.1038/srep15145

Yang, J., Zhou, W., Zhu, J., Wu, Y., Xu, L., Wang, Y., et al. (2019). Circulating ectodysplasin A is a potential biomarker for nonalcoholic fatty liver disease. *Clin. Chim. Acta* 499, 134–141. doi: 10.1016/j.cca.2019.09.009

Zeng, S., Zhao, X., Xu, L., Yang, D., Chen, L., and Xu, M. (2019). Apocynum venetumApoptosis induction effect of polyphenol on human U87 glioma cells via NF-κB pathway. *Fut. Oncol.* 15, 3723–3738. doi: 10.2217/fon-2019-0381

Zhang, Y., Tomann, P., Andl, T., Gallant, N., Huelsken, J., Jerchow, B., et al. (2009). Reciprocal requirements for EDA/EDAR/NF-kappaB and Wnt/beta-catenin signaling pathways in hair follicle induction. *Dev. Cell* 17, 49–61. doi: 10.1016/j.devcel.2009.05.011

Zhao, M., Zhang, Y., Li, J., Li, X., Cheng, N., Wang, Q., et al. (2018). BIMHistone deacetylation, as opposed to promoter methylation, results in epigenetic silencing and resistance to EGFR TKI in NSCLC. *Oncol. Lett.* 15, 1089–1096. doi: 10.3892/ol.2017.7411

Zouvelou, V., Luder, H., Mitsiadis, T., and Graf, D. (2009). Deletion of BMP7 affects the development of bones, teeth, and other ectodermal appendages of the orofacial complex. *J. Exp. Zool. B. Mol. Dev. Evol.* 312B, 361–374. doi: 10.1002/jez.b.21262

Zurlo, F., Larson, K., Bogardus, C., and Ravussin, E. (1990). Skeletal muscle metabolism is a major determinant of resting energy expenditure. *J. Clin. Invest.* 86, 1423–1427. doi: 10.1172/jci114857

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frontiers in Physiology

ORIGINAL RESEARCH
published: 20 January 2022
doi: 10.3389/fphys.2021.736244

Acute/Chronic Responses of Combined Training on Serum Pro-thermogenic/Anti-inflammatory Inducers and Its Relation With Fed and Fasting State in Overweight Type 2 Diabetic Individuals

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OPEN ACCESS

Edited by:
Guilherme Fleury Fina Speretta, Federal University of Santa Catarina, Brazil

Reviewed by:
Roberto Codella, University of Milan, Italy
Tom L. Broderick, Midwestern University, United States

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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: 04 July 2021
Accepted: 21 December 2021
Published: 20 January 2022

Citation:
Bonfante ILP, Duft RG, Mateus KCdS, Trombeta JCdS, Finardi EAR, Ramkrapes APB, Brunelli DT, Mori MAdS, Chacon-Mikahil MPT, Velloso LA and Cavaglieri CR (2022) Acute/Chronic Responses of Combined Training on Serum Pro-thermogenic/Anti-inflammatory Inducers and Its Relation With Fed and Fasting State in Overweight Type 2 Diabetic Individuals. Front. Physiol. 12:736244. doi: 10.3389/fphys.2021.736244

Concentrations of pro-thermogenic/anti-inflammatory inductors are influenced by fed/fasting, sedentary/trained states, and metabolic pattern. However, there is a lack of information on the interactions of these conditions, especially in humans. Thus, the present study aimed to evaluate the chronic and acute training responses as well as the fed/fasted states of serum pro-thermogenic/anti-inflammatory inducers in overweight type 2 diabetics individuals. Fifteen individuals with type 2 diabetes [body mass index (BMI): 29.61 ± 3.60 kg/m^2; age: 50.67 ± 3.97 years] participated in the study. In the pre- and post-experimental periods, baseline clinical parameters analyses were performed. Pro-thermogenic/anti-inflammatory inductors were evaluated pre/post-baseline and before, shortly after, and after 30′ and 60′ in the first and last sessions of a 16-week combined training (CT) period. These inducers were also compared for fasting and feeding before and after the training period. CT has improved baseline physical fitness, metabolic pattern, and it has also increased interleukin (IL)33 and FNDC5/irisin. In the first training session, there was a decrease in IL4, IL13, and IL33, besides an increase in FNDC5/irisin, and natriuretic peptides. In the last training session, there was an increase in natriuretic peptides and bone morphogenic protein 4 (BMP4). Differences in responses between the first and last training sessions were observed at certain post-session times for IL4, IL33, and natriuretic peptides, always with higher concentrations occurring in the last session. In evaluating the area under the curve (AUC) of the first and last training session, FNDC5/irisin, natriuretics peptides, and meteorin-like showed increased areas in the last training session. The pre-training fed state showed

an increase in IL4 and IL33, while in fasting there was an increase in meteorin-like, natriuretic peptides, and FNDC5/irisin. In the post-training, IL4, IL13, and IL33 were increased in the fed state, while meteorin-like, natriuretic peptides, and FNDC5/irisin remained increased in the fast. Adaptation to physical training and a better metabolic pattern favor an improvement in the acute secretory pattern in part of pro-thermogenic and anti-inflammatory substances analyzed. The fed and fasting states also interfere differently in these substances, where fasting interferes with the increase of myokines, while the fed state induces an increase in interleukins.

Clinical Trial Registration: [http://www.ensaiosclinicos.gov.br/rg/RBR-62n5qn/], identifier [U1111-1202-1476].

Keywords: brown adipose tissue (BAT), browning of white adipose tissue (WAT), inflammation, interleukins, metabolism, myokines, type 2 diabetes

INTRODUCTION

The induction of adipocyte thermogenesis is effective to treat cardiometabolic diseases such as obesity and type 2 diabetes (T2D) since it is associated with increased energy expenditure, weight loss, improved insulin sensitivity, and glucose uptake (Villarroya and Vidal-Puig, 2013).

Physical exercises and certain dietary states/patterns are physiological strategies that show evidence of the thermogenic and mitochondrial capacity of adipocytes stimulation *via* sympathetic or hormonal signaling mediated by serum pro-thermogenic products that are also associated with cardioprotective and anti-inflammatory effects (Bostrom et al., 2012; Villarroya and Vidal-Puig, 2013; Fabbiano et al., 2018).

The stimulus for the secretion of these pro-thermogenic products induced by physical exercise or dietary state/pattern is due to factors as energy need, hypoxia, and metabolic/thermal stress, which stimulate the secretion of catecholamines and other pro-thermogenic substances such as interleukins 4 (IL4), IL13 and IL33, fibroblast growth factor 21 (FGF21), irisin, natriuretic peptides, meteorin-like and bone morphogenic protein 4 (BMP4), and BMP7 (Tseng et al., 2008; Schulz et al., 2013; Qian et al., 2013; Kajimura and Saito, 2014; Lee et al., 2014; Rao et al., 2014; Brestoff et al., 2015; Li et al., 2015; Modica et al., 2016; Hoffmann et al., 2017). However, most of these findings related to pro-thermogenic factors are based on experimental studies with animal or *in vitro* models, not yet been comprehensively evaluated in humans, even more so with the interaction between physical exercise, dietary status, and worsened metabolic state like T2D.

Certain studies show that in physical exercises the effects are not consensual on some of these pro-thermogenic factors, which seems to vary according to the moment of the analysis (right after the practice or baseline) and the level of physical fitness of the analyzed subject (sedentary versus trained) (Huh et al., 2012, Huh et al., 2014; Petriz et al., 2017). A single bout of low exercise intensity already induces at least moderate favorable changes in glycemic and lipidemic profiles after a certain breakfast pattern, for example (Benedini et al., 2019). In addition, certain catabolic and inflammatory markers showed differences between acute and chronic responses to functional and resistance exercises (Faelli et al., 2020). In food, factors such as the postprandial period (Din et al., 2018), caloric restriction/fasting (Roca-Rivada et al., 2013; Fabbiano et al., 2016, 2018) also appear to influence the secretion of some of these substances and products related to the energy balance, especially in the short term (Benedini et al., 2011).

Other factors that seem to influence the combination of these markers are the metabolic pattern, the presence of overweight, T2D, and insulin resistance, for example, which is associated with lower baseline concentration of some of these pro-thermogenic factors such as irisin or natriuretic peptides (Du et al., 2016; Verboven et al., 2017). These effects are probably related to the sedentary lifestyle and positive energy balance present in these diseases, which decreases the secretion of pro-thermogenic/anti-inflammatory products, especially myokines (Gleeson et al., 2011; Eckardt et al., 2014). Additionally, these chronic diseases are associated with low-grade chronic inflammation, characterized by increased production of inflammatory inducers and decreased anti-inflammatory products (Gleeson et al., 2011).

Based on the pro-thermogenic function and anti-inflammatory effects of these cited substances, in addition to the lack of information related to the influence of the fed/fasting, sedentary/trained states and metabolic pattern on its concentrations, as well as the interaction between these conditions in humans, the aim of the present study was to evaluate the acute/chronic effects of training on pro-thermogenic/anti-inflammatory serum inducers in type 2 overweight diabetics and the influence of the fed/fasting state on these markers. We hypothesized that an exercise session could induce increases in the pro-thermogenic/anti-inflammatory serum inducers, but that metabolic and physical fitness improvement provided by the adaptation to physical training would induce an even greater increase in the secretion of these products. In addition, we also assume that fasting and being fed in different ways influence these pro-thermogenic/anti-inflammatory products according to their characteristics (cytokines × myokines) and that adaptation to training could mitigate these differences.

MATERIALS AND METHODS

Volunteers

This study presents the secondary results (acute training and fed/fasted state responses on pro-thermogenic/anti-inflammatory; and the association between these results and basal metabolic/clinical markers) from the randomized controlled trial UTN: U1111-1202-1476[1]. The primary results (molecular and thermogenic profile of adipocytes; clinical and thermogenic basal markers) were presented in a randomized controlled trial (Bonfante et al., under review). The study was approved by the Research Ethics Committee of the University of Campinas Medical School and was based on the principles of the Declaration of Helsinki.

The study inclusion criteria were: diagnosis of T2D; age between 40 and 60 years old, body mass index (BMI) from 25 to 35 kg/m^2; non-active life habit; not having regularly participated in any training program and/or executed any systematized diet during the previous 12 months of study; to have availability to participate in the first and last training sessions protocols. The exclusion criteria were: arrhythmia or cardiac ischemia, coronary artery disease, severe arterial hypertension, chronic obstructive pulmonary disease, anemia, uncontrolled hypothyroidism, limiting bone-joint diseases; general and or specific serious complications of T2D; not be approved in exercise electrocardiogram; to use some medicine as exogenous insulin, thiazolidinediones, beta-blockers, anticoagulants, anti-inflammatories. The medicines used are shown in **Table 1**. No changes were made in the medicines used (types and dosages) during the experimental period. As criteria for discontinuity, were adopted: the lack of motivation or willingness of the volunteer to attend training sessions; attendance at training sessions below 85% and/or more than three consecutive absences; other risks that could occur to the volunteers even after clinical release.

The clinical and biochemical data have already been presented in the primary outcomes of the trial, with a comparative analysis between-group (training and control) versus time and a total of 17 subjects per group. All subjects were recruited through publicity in the media and university. Recruitment was set to the primary and secondary objectives of the research project. In the first round, 363 volunteers were screened; 297 were excluded due to the inclusion/exclusion criteria. The 66 volunteers included were randomly assigned to the combined training group (CTG) (n = 41) or control group (CG) (n = 25). More subjects were included in the CTG due to the greater risk of discontinuation. The randomization number sequence was created using Excel 2019 (Microsoft, Redmond, WA, United States) with a 1:1 ratio allocation. The randomization was performed by a researcher not involved in this project to avoid selection bias. During experimental protocol time (training or control), 30 volunteers were excluded due to failure to adhere to the protocol (job change, city change, difficulty to go to the evaluations, and/or to train for lack of time or money and other particular reasons). Another two volunteers were excluded for medical reasons, uncontrolled hypothyroidism and anemia, identified in laboratory tests. Thus, 34 volunteers completed the study, 17 in the CTG (10 women and 7 men) and 17 in the CG (8 women and 9 men). Of the 17 volunteers in the CTG, 15 (9 women/6 men) underwent first and last training sessions protocols in order to present training adaptations and differences between sedentary and trained states. The complete flowchart is shown in **Figure 1**. The reason not all participants underwent first and last training sessions was the time limitation of the volunteers because of professional/work reasons. In addition, due to technical limitation (error in performing two enzyme-linked immunosorbent assay (ELISA) kit plates, one of FNDC5/irisin and one of the natriuretic peptides), we had an "n" of 8 subjects (4 men and 4 women) for FNDC5/irisin and an "n" of 12 individuals (5 men and 7 women) for natriuretic peptides. A similar male to female ratio was maintained for FNDC5/irisin and natriuretic peptides according to the distribution of our samples on the analysis plates.

A power analysis was conducted *a priori* and *a posteriori* with G*POWER 3.1 software (Universitat Kiel, Germany). *A priori*, we based initially on waist circumference, since the amount of abdominal fat is one of the main factors related to T2D and interfere with primary outcomes analyzed in the first study. We based on a study that aimed to analyze the combined training effects in middle-aged subjects, type 2 diabetics, which presented the following waist values in (cm): (pre 118.6 ± 11.6 and after 112.6 ± 11.9) (Lucotti et al., 2011). A total of 28 participants was obtained (14 per group, training versus control), considering a statistical design of the F test (2 × 2 ANOVA for repeated measures), calculation of the f effect size of 0.53, type I error (α) 5%, a correlation coefficient of 0.5, and test power size of at least 95%. Additionally, for the present study design, we based pre–post acute effects of exercise on irisin levels from a study with healthy young men, who performed one session of endurance and another of resistance exercise (Nygaard et al., 2015). Although we analyzed several markers in the first and last training sessions, we chose irisin because it is one of the main circulating inducers associated with exercise, thermogenesis, and metabolic health (Bostrom et al., 2012; Lee et al., 2014; Otero-Diaz et al., 2018). The study used presented the following irisin values in (ng/ml): (endurance exercise pre

[1]http://www.ensaiosclinicos.gov.br/rg/RBR-62n5qn/

TABLE 1 | Medicine used by subjects.

	Number of subjects using the medicine
Sulfonylurea	6
Metformin	15
SGLT2 blockers	2
DPP-4 inhibitors	3
Lipid-lowering drug therapy	5
Antihypertensive	8
Antidepressant	2
Levothyroxine	2
Vitamin D	1
Calcium	1

SGLT2, sodium-glucose cotransporter 2; DPP4, dipeptidyl peptidase-4.

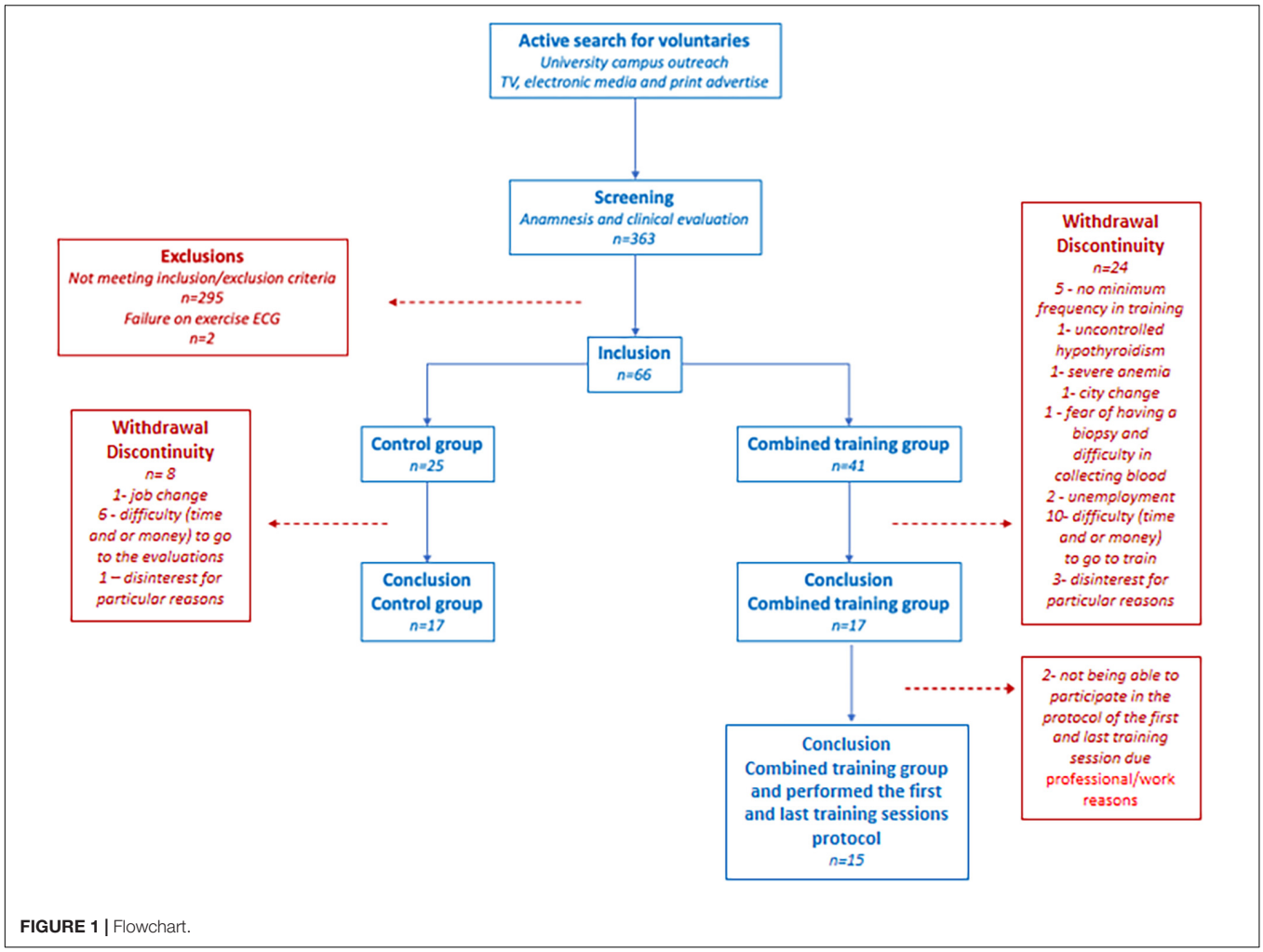

FIGURE 1 | Flowchart.

382 ± 41 and after 459 ± 51; resistance exercise pre 355 ± 50 and after 437 ± 56) (Nygaard et al., 2015). A total of six subjects would be needed based on endurance exercise and eight subjects based on resistance exercise, considering: a statistical design of the *F* test (one-way ANOVA for repeated measures, within factors); calculation of the *f* effect size = 1.66 (endurance exercise) and 1.54 (resistance exercise); type I error (α) 5%, correlation coefficient of 0.5; and test power size of at least 95%. Still using this data of Nygaard et al., 2015, however, for a statistical design of the *F* test (2 × 2 ANOVA for repeated measures), the results were a sample of six subjects per group (first session group versus last session group) based on both, resistance and endurance data. Finally, we calculated the posteriori power of FNDC5/irisin results from eight participants (first training session). This calculation was based on the statistical test used in the study, an alpha significance level of 0.05, an effect size of 0.75 from the pre and post 60′ values (post 60′ was the higher value of time course) of the means and standard deviation of FNDC5/irisin levels. As a result, we observed a beta power of 0.998 for a statistical design of the *F* test (2 × 2 ANOVA for repeated measures) and 0.997 (one-way ANOVA for repeated measures, within factors). These beta powers is up from the normally stipulated power of 0.80, which is usually used as a minimum required to characterize a sample as capable of detecting a difference in a given population.

Experimental Design

After a media call by volunteers, structured anamnesis and physical activity questionnaires were applied to those interested in participating in the study to meet the volunteers and evaluate the inclusion and exclusion criteria. Those who met the inclusion criteria underwent clinical and medical evaluations.

Those approved to participate were familiarized with the location, tests, and equipment used from visiting the premises where the assessments were carried out, as well as explaining and experiencing the procedures and tests that would be used.

Clinical evaluation, functional evaluation tests, collection of biological material, and training practice were performed under conditions of spontaneous breathing of atmospheric air in a room with an average ambient temperature of 23°.

Initially, the subjects underwent baseline assessments: anthropometric, body composition, functional [cardiorespiratory, muscle strength, resting metabolic rate (RMR)], nutritional assessment, blood collection, and hemodynamic. All of these initial assessments lasted for

around 2 weeks, always with an interval between 48 and 72 h between assessments that required abstinence from physical exercises or previous stress tests. Also, it was recommended before each assessment to abstain from alcohol for 24 h, caffeine, or any stimulating drink for 12 h. For blood collection and RMR, fasting was still recommended for 12 h. All assessments were repeated after 16 weeks of training following the same initial recommendations. Before the last training session in the 16th week of training, VO_2max and 12-maximum repetition tests were performed. The participants underwent two sessions of familiarization with the equipment and training protocol before strength assessments. The experimental design is detailed in **Figure 2A**.

After these baseline evaluations, participants performed the pre-training session, after having a standard breakfast (20 g of Quaker® oats (Quaker Brazil, Guarulhos, Brazi), 1 pot of 90 g Nestlé® light Greek yogurt (Nestlé Brazil, São Paulo, Brazil), 1 packet of whole Nesfit® cookies (Nestlé Brazil, São Paulo, Brazil) and banana) in their homes after 10–12 h of fasting. To carry out this pre-session, the subjects followed the following recommendations: take the medication normally; wear appropriate clothes to exercise; eat only a standard breakfast 1 h and 30 min before the experimental session and; abstain from caffeine or any stimulating drink for 12 h and alcohol for 24 h and no exercise during the 48–72 h before the session. The volunteers were instructed to make a note of their previous dinner and try to repeat it in the last-training session. Before (after 10 min sitting at rest), immediately after, 30 min and 1 h right after the session, blood samples were taken using an intravenous catheter. The catheter was inserted before the training session and remained throughout the exercise session and in the resting period after the end of the exercise. The entire procedure followed before and during the first session was performed again in the last training session. For more details vide **Figure 2B**. The post-session blood collection times were based on time course studies of irisin secretion by exercises and on the idea that the analyzed analyte secretion is stimulated by the energy needed. This entire protocol was repeated in the last training session. During the pre- and post-training sessions and in the subsequent resting period, the participants could use the bathroom and hydrate by drinking water freely.

Evaluation Protocols

Physical Activity Level and Exercise Electrocardiogram

Baecke and IPAQ questionnaires were applied to assess the level of habitual physical activity of volunteers (Nyssen et al., 2013). The questionnaires showed that the volunteers did not perform systematic physical activity in the period before the study and had an average weekly physical activity time of 105.50 ± 66.03 min. Volunteers who met the inclusion criteria underwent an exercise electrocardiogram (Bruce protocol) (Brunelli et al., 2015).

Anthropometry and Body Composition

Bodyweight and height were measured on a platform scale (Filizolla®, São Paulo, Brazil) with a coupled stadiometer. From the weight and height data, BMI was calculated. Neck, waist, and hip circumferences were measured using a measuring tape with an accuracy of 1 mm and based on standard anatomical references for these regions.

Body composition was assessed by whole-body plethysmography (air displacement plethysmography, BOD POD^R – COSMED USA, Inc., Concord, CA, United States), based on the criteria described in the equipment manual and previously used (Gomez-Ambrosi et al., 2011).

Muscle Strength

After familiarization, the 12-maximum repetition (adapted) test was performed to determine the load of each exercise used in the strength training protocol (Abdul-Hameed et al., 2012). Three to 6 attempts were made, with a 2- to 3-min interval to obtain the 12 maximum repetitions.

The maximum repetition test (1RM) was also applied (Brunelli et al., 2015), which consisted of assessing the strength of the lower and upper limbs using the leg press and bench press exercises, respectively.

In the pre-trial period, this test was performed twice (test and re-test), the first being used to familiarize and minimize the influence of neural adaptations gains and the learning effect, while the second was used as a reference value. At the post, there was only one re-evaluation.

Cardiorespiratory Assessment, Heart Rate, and Blood Pressure

Baseline heart rate and blood pressure were measured after 5 min of rest (seated), using a cardio frequency meter and a mercury sphygmomanometer and stethoscope. The cardiorespiratory assessment was performed using a progressive effort protocol on a treadmill (Quinton, model TM55, United States), with a continuous collection of expired gases breath to breath (CPX Ultima, MedGraphics, United States). The cardiorespiratory fitness verified by the maximum oxygen consumption (VO_2max) was determined by the average values of the last 30 s of the test. The criteria used for stopping the cardiorespiratory assessment were based on the perceived exertion equal to 20 (Borg scale 6–20), Respiratory Exchange Ratio (RER > 1.1), maximum heart rate within 10 beats of the age-appropriate reference value, and inability to maintain the speed on a treadmill (Brunelli et al., 2015).

Rest Metabolic Rate

The determination of the RMR was performed using oxygen consumption and carbon dioxide production, using indirect open circuit calorimetry by the gas analysis system (Ultima CPX, MedGraphics, United States) which was calculated in daily values (kcal/day) by the Weir equation (Bonfante et al., 2017).

For this test, the volunteers wore a face mask connected to the gas analyzer, remaining silent, in the supine position, avoiding moving and not sleeping for 30 min, so that breath by breath was captured. The gas analyzer was calibrated before each test. The first 10 min were not considered for RMR calculation, because of stabilization of the physiological variables at resting state. The last 5 min also were not considered due to the evaluator's movement in the room. In addition, we oriented the participants to make

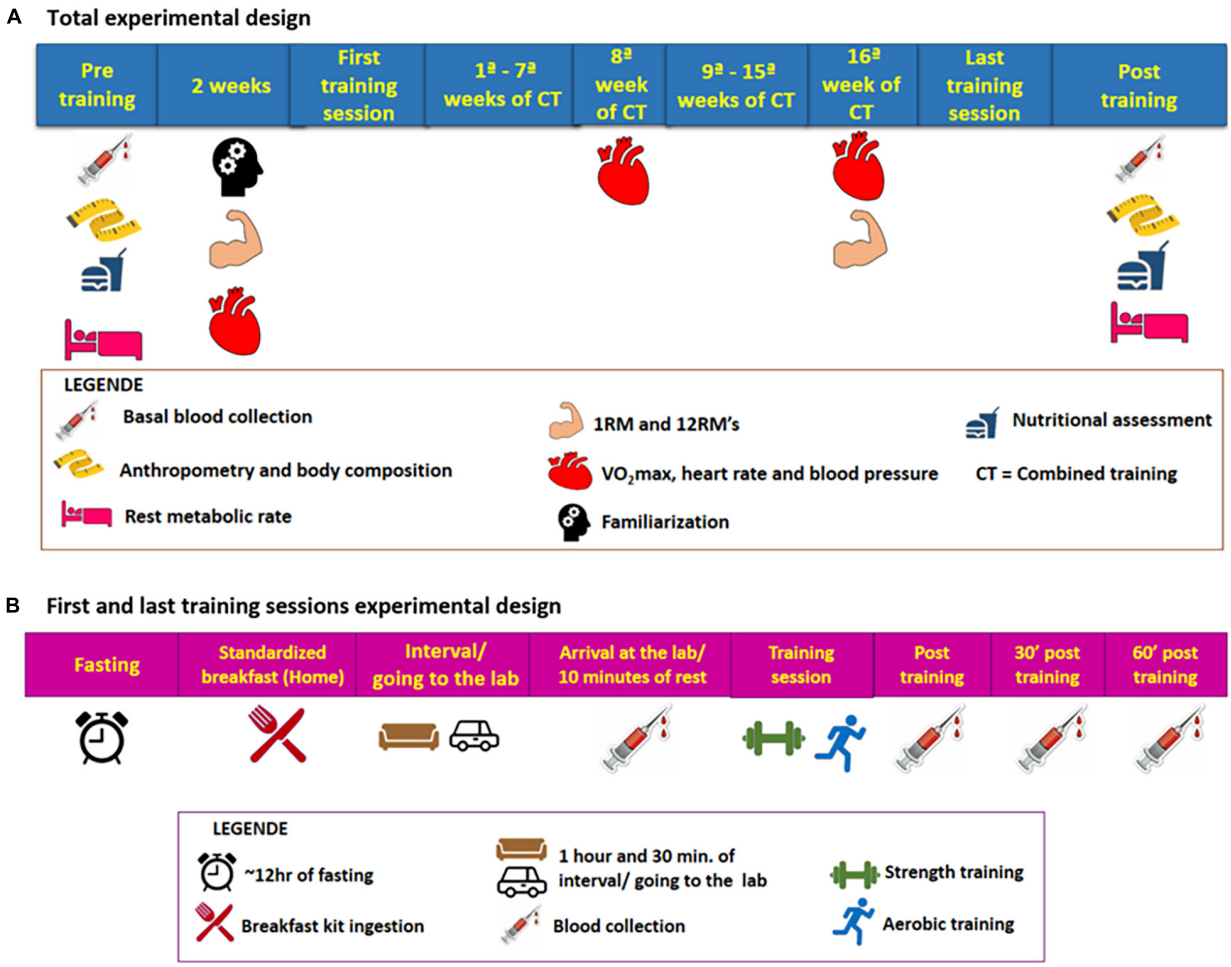

FIGURE 2 | (A) Total experimental design (chronic design). **(B)** First and last training sessions experimental design (acute design).

their locomotion by motor vehicle to the testing site to ensure minimal physical activity before RMR determination.

Blood Samples

Blood samples were collected at baseline-post 16-week experimental protocol, and in first and last training sessions (before, immediately after, post 30′, post 60′ of training sessions). For the food status, the baseline-post 16-week experimental protocol was used to assess the influence of fasting, while the moment pre of first and last sessions' were utilized to characterize the feeding pattern.

Baseline blood samples (approximately 40 ml) were collected from the antecubital vein in a dry vacuum tube with anticoagulant (EDTA). All of them were always performed at the same time (between 7:00 and 9:00 am), after a period of abstinence from exercising between 48–72 and a 12-h fast.

The collections of the training sessions (between 7:00 am and 10:00 am), 2 h after eating a standardized breakfast. Blood samples were collected in one dry vacuum tube and one tube with anticoagulant (EDTA) (one tube each for the time of collection). Both blood collections were performed using an intravenous catheter and a PRN adapter plug inserted into the brachial vein at the height of the antecubital fossa, which remained throughout the exercise session and in the posterior rest period.

Some samples were readily analyzed and others were stored in a −80° freezer after being centrifuged for further analysis.

Biochemical Analyses

The plasma glucose analysis was performed in the GOD-Trinder method. The serum was used to analyze: insulin, C peptide, TSH (chemiluminescence method), triglycerides, total cholesterol (enzyme-trind method), high-density lipoprotein (HDL) (accelerator-selective detergent method), very low-density lipoprotein (VLDL), and low-intensity lipoprotein (LDL) (Friedewald formula method). Using whole blood were made glycated hemoglobin (high-performance liquid chromatography method) and blood cell counts (automated microscopy method). From the results of glucose and Insulin, the HOMA-IR index was calculated using the formula (Bonfante et al., 2017).

The concentrations of cardiac natriuretic peptides (*Aviva Biosystems*, United States) IL4, IL13, IL33, FGF 21, BMP4, BMP7 meteorin-like (*R&D System*, United States), and FNDC5/irisin

(*US Biological*, United States) were performed by the method ELISA according to the manufacturers' specifications.

Nutritional Assessment

The volunteers were instructed to maintain their eating habits throughout the study. To ensure that this recommendation was being followed, the volunteers were routinely alerted to this aspect and even filled out food records (FRs) before and after the experimental period. They were asked to report in the FR all the food and drinks eaten during the established days (different and non-consecutive days; two weekdays and one weekend day to have an average of the three recalls) (Brunelli et al., 2015).

The FR was analyzed semi-quantitatively delimiting the number of portions ingested per day of carbohydrates, raw and cooked vegetables, fruits, milk and dairy products, meat, legumes, and energy supplements. Subsequently, these portions had their amounts of macronutrients (in grams) calculated and the total caloric intake, based on the pre-established portions and formulas (Brunelli et al., 2015).

Combined Training Protocol

The 16 weeks of combined training consisted of sessions with 5 min warm-up on an exercise bike and subsequent performance of the resistance training (RT), followed by the aerobic training (AT) in the same session, divided into two stages and performed on three alternate days a week (Mondays, Wednesdays, and Fridays). The entire volume and intensity of the training followed the recommendations for subjects with T2D (Colberg et al., 2010, 2016).

In stage 1, the RT was composed of a linear periodization with 10 exercises (7–8 per day) with priority to work large muscle groups, which are: leg press, knee extension, knee flexion, bench press, high pull (performed in three training days off a week) and barbell curls, triceps pulley, smith machine shoulder press, extension/flexion of the calf and upper abdominal (performed one or two training days a week). These exercises had 3 sets of 12 submaximal repetitions and a 1-min pause between sets, which took approximately 37–42 min to perform the RT. The ordering of the exercises was alternated by segment in this phase. Then the participants moved to the treadmill and occasionally to the athletics track, where they performed 35 min of AT with walking/trotting/running (according to the volunteer's physical fitness level in the VO_2max test), with intensity variation between 50 and 65% of VO_2max., being 3.5 min at 50–55% of VO_2max., 14 min at 55–60% of VO_2max., 14 min at 60–65% of VO_2max., and 3.5 min between 50 and 55% VO_2max (Colberg et al., 2010, 2016).

In stage 2, the RT session was performed with the same exercises and series as stage 1; however, with 10 submaximal repetitions and a 1-min and 15-s pause, totaling approximately the same 39–42 min as stage 1. This was done to maximize the load increase by the submaximal repetition test based on the principle of interrelation between volume/intensity. With the decrease in volume, there was an increase in the load when compared to the previous stage. In this stage, the ordering of the exercises was performed by the initial performance of the upper muscle segment, followed by the lower or vice versa (it is recommended to alternate the initial segment at each session). For AT there was the same training pattern, but there was an adjustment in the training intensity zones (a new VO_2max test was performed after the 8th training week). AT intensities were between 50 and 70% of VO_2max, with 3.5 min at 50–60% of VO_2max, 14 min at 60–65% of VO_2max., 14 min at 65–70% of VO_2max., and 3.5 min between 50 and 60% VO_2max (Colberg et al., 2010, 2016).

Throughout the program, the intensity of the AT was controlled by the speed associated with the percentage of VO_2max. predicted provided at each stage of training, besides verification of heart rate and subjective perception of effort (Borg scale).

Regarding adaptation to AT (decreased HR and or perceived exertion below or equal to "relatively easy/easy") before the change in training intensity by the intermediate aerobic reassessment of 8 weeks or during any training period, 0.2 km/h were increased at each intensity of aerobic exercise.

In the RT, load readjustments were carried out every 2 weeks in the first month and then weekly, with the application of submaximal repetition tests. With this procedure, it was observed a progressive increase in training overload and loads that were for most volunteers between 50 and 75% of 1RM (Colberg et al., 2010, 2016).

First and Last Training Sessions

In the first and last sessions of the training program, the general characteristics of the proposed Combined training (CT) were followed with some modifications to avoid major overload in the pre-session by the untraining volunteers at this time. In RT, the priority of working in large muscle groups was maintained (seven exercises), which were performed by 2 sets of 12 adapted submaximal, a pause of 1 min between series and alternating exercise order by segment and execution speed of 2 s for the eccentric movement and 2 s in the concentric movement, having a total duration of approximately 25 min. Afterward, 25 min of AT (walking/trotting/running) was performed on a treadmill alternating the intensity by 5 min at 40–45% of the VO_2max., 7.5 min at 45–50% of the VO_2max., 7.5 min at 50–55% of the VO_2max., and 5 min between 40 and 45% of the VO_2max. Before the start of the session, a 5-min warm-up was performed on an exercise bike. The same characteristics of the first training session were maintained in the last session, however, the intensity of the RT and AT were adjusted according to the results of the previous VO_2max and 12-maximum repetition tests, which were performed pre-trial period and before the last training session. The sessions were supervised using the BORG scale and the heart rate monitor (Colberg et al., 2010, 2016).

Statistical Analysis

Initially, the Kolmogorov–Smirnov test was applied to test data normality. In the case of non-parametric data, transformation into log Ln was applied.

For the comparison between the data of the pre and post in the same condition (baseline clinical and biochemical parameters; pro-thermogenic/anti-inflammatory inducers in food status), Student's *t*-test was applied for dependent samples.

Student's dependent *t*-test was used to compare pre- and post-training moments in isolation of fed versus fasting state. To compare pre- and post-training food status together (pre–post fasting versus pre–post fed) was applied repeated measures two-way ANOVA or analysis of covariance (ANCOVA). In ANOVA two-way, whenever a significant *F* value was obtained (group × time significant interaction value "*P*"), Tukey's *post hoc* test was applied.

In training sessions results, primarily, repeated measures ANOVA (one-way) was applied to compare the time effect separately in the first and last training sessions. Whenever a significant *F* value was obtained in ANOVA one-way (time significant value "*P*"), Tukey's *post hoc* test also was applied. Posteriorly, repeated measures ANOVA two-way and Tukey's *post hoc* (if applicable) were performed to determine significant differences in group × time between first and last training sessions comparisons. In addition, the area under the curve (AUC) was calculated by trapezoidal approximation for each analyte analyzed in the first and last training session. The comparison between the AUC of the first and last training sessions was made by student's *t*-test.

Pearson's correlation coefficient was also applied to assess the correlation between Δ% basal serum pro-thermogenic/anti-inflammatory inducers and clinical variables.

Results are presented as means ± standard deviation (**Tables 2**, **3**) or mean ± standard error (**Figures 3**, **4**) or mean (**Figure 5** – AUC). The level of significance used was $P < 0.05$. All analyses were performed using Statistica 6.0 software.

RESULTS

Combined Training Improved Clinical Markers and Metabolic Status

As expected, CT induces improvement in several clinical variables associated with body composition and biochemical markers related to glycemic and lipid metabolism. CT protocol has reduced neck, waist, and hip circumferences, fat mass, resting heart rate, glucose, glycated hemoglobin, triglycerides, and VLDL-c. Furthermore, CT increased hemoglobin, Hematocrit, lean mass, muscle strength (leg press and bench press exercises), VO_2max, and HDL-c (**Table 2**). No changes were observed in food behavior (**Table 2**) and white blood cells (data not shown).

It is important to note that these results (except blood cell count) were presented in a primary randomized controlled trial (Bonfante et al., under review). In a group/time comparison design, the results are the same as here observed for neck and waist circumferences, fat mass, resting heart rate, glucose, glycated hemoglobin, triglycerides, VLDL-c, lean mass, muscle strength, and VO_2max.

Combined Training Effects on Pro-thermogenic/Anti-inflammatory Markers Concentrations

Combined training increased pre- and post-training baseline IL33 ($P = 0.01$) and FNDC5/irisin ($P = 0.04$) on fast (**Table 3**). No changes were observed in pre- and post-training fed (**Table 3**). In a group/time comparison design of the primary study, IL33 and FNDC5/irisin presented the same pattern of behavior in the comparison between Δ% of the training and CG.

Because of these CT effects on fasting pro-thermogenic/anti-inflammatory markers concentrations and clinical/metabolic status, we performed correlations between the Δ% of changing of these variables. As a result, positive correlations were observed between IL-4/eosinophils; IL-13/lymphocytes ($r = 0.79$, $P < 0.01$); FNDC5-irisin/HDL-c ($r = 0.83$, $P = 0.02$); meteorin-like/basophils ($r = 0.45$, $P = 0.04$) and between bench press ($r = 0.53$, $P = 0.04$); FGF21/VO_2max ($r = 0.63$, $P = 0.01$) and leg press ($r = 0.63$, $P = 0.01$); and BMP7/eosinophils ($r = 0.81$, $P = 0.02$). Negative correlations were also observed between IL4/hip circumference ($r = -0.62$, $P = 0.01$); IL13/glucose ($r = -0.78$, $P = 0.03$); FNDC5-irisin/resting heart rate ($r = -0.64$, $P < 0.01$); meteorin-like/diastolic blood pressure ($r = -0.53$, $P = 0.04$), neck circumference ($r = -0.53$, $P = 0.04$); FGF21/insulin ($r = -0.60$, $P = 0.01$), C peptide ($r = -0.54$, $P = 0.03$); and BMP4/waist circumference ($r = -0.81$, $P = 0.02$).

Baseline Fasting/Fed Pro-thermogenic/Anti-inflammatory Markers Concentration

The data about serum thermogenesis inductors in the fasting and fed states before training are shown in **Table 3**. IL4 ($P = 0.01$) and IL33 ($P = 0.02$) exhibited a decrease in fasting when compared to feeding, while natriuretic peptides ($P = 0.01$), meteorin-like ($P < 0.001$), and FNDC5/irisin ($P = 0.01$) exhibited an increase in fasting (**Table 3**).

Interactions Between Training and Fasting/Fed on Pro-thermogenic/Anti-inflammatory Markers Concentration

After training, IL4 ($P = 0.001$), IL13 ($P = 0.02$), and IL33 ($P = 0.05$) presented a decrease in fasting when compared to feeding, while natriuretic peptides ($P < 0.01$), meteorin-like ($P < 0.001$), and FNDC5/irisin ($P = 0.04$) remained increased in fasting (**Table 3**).

In pre/post responses between fasting and fed status (conditions × time comparison) were observed differences only for IL33 (**Table 3**), probably occurred due to the increase observed between pre- and post-training fasting.

Effects of the First and Last Training Session: Sedentary Versus Trained Status

Based on evidence that exhibits the acute secretion of pro-thermogenic/anti-inflammatory inducers by exercise, as well as the differences in the responses according to the subjects' physical fitness, we analyzed the time-course serum responses of these inductors' outcomes in the first and last training session. In the first training session IL4 ($P = 0.03$), IL13 ($P = 0.02$), and IL33 ($P = 0.03$) show a decrease in the post 60′ when compared to the pre-training moment in the pre-session (**Figures 3A–C**). In

TABLE 2 | Clinical parameters of volunteers.

	Pre	Post	*P*
Female/male	9/6	–	–
T2D diagnosis (years)	5.56 ± 2.55	–	–
Age (years)	50.67 ± 3.97	–	–
Height (cm)	1.67 ± 0.07	–	–
Weight (kg)	83.62 ± 13.41	83.05 ± 13.41	0.08
BMI (kg/m^2)	29.61 ± 3.60	29.41 ± 3.61	0.08
Neck circumference (cm)	39.63 ± 4.47	38.83 ± 4.50[a]	< 0.0001
Waist circumference (cm)	96.86 ± 10.20	94.33 ± 10.04[a]	< 0.0001
Hip circumference (cm)	106.26 ± 6.22	104.46 ± 5.82[a]	0.01
Lean mass (kg)	52.94 ± 10.00	54.12 ± 10.90[a]	< 0.001
Fat mass (%)	35.79 ± 5.21	34.37 ± 5.41[a]	< 0.001
VO_2max. (ml/kg/min)	21.48 ± 4.06	23.59 ± 4.21[a]	< 0.01
Leg press 1 RM (kg)	194.80 ± 78.22	245.33 ± 88.62[a]	< 0.0001
Bench press 1RM (kg)	33.73 ± 23.27	40.60 ± 22.73[a]	0.02
RMR (kcal/day)	1328.21 ± 347.16	1442.09 ± 396.96	0.08
Systolic BP (mmHg)	117.30 ± 14.40	113.10 ± 14.70	0.14
Diastolic BP (mmHg)	74.40 ± 11.20	73.20 ± 9.00	0.17
Resting heart rate (bpm)	81.26 ± 11.87	75.26 ± 11.13[a]	0.03
Carbohydrate consumption (g/day)	311.80 ± 95.03	318.15 ± 93.03	0.77
Lipid consumption (g/day)	55.30 ± 20.63	54.14 ± 16.58	0.77
Protein consumption (g/day)	71.2 ± 21.82	79.55 ± 21.63	0.36
Total calories (kcal/day)	1661.28 ± 473.13	1713.11 ± 483.98	0.97
Erythrocytes (mi/mm^3)	4.81 ± 0.48	4.90 ± 0.37	0.08
Hemoglobin (g/dl)	13.7 ± 1.19	14.06 ± 1.15[a]	0.04
Hematocrit (%)	40.26 ± 2.86	41.71 ± 2.31[a]	0.01
C peptide (ng/ml)	2.68 ± 1.07	2.38 ± 0.71	0.25
Insulin (μU/ml)	14.25 ± 7.93	12.98 ± 6.09	0.11
Glucose (mg/dl)	141.40 ± 34.34	121.20 ± 14.25[a]	0.02
HbA1c in mmol/mol/(%)	63.5/(7.96 ± 2.42)	55.2/(7.20 ± 1.55)[a]	0.04
HOMA-beta (%)	72.86 ± 44.71	88.13 ± 62.18	0.23
HOMA-IR (%)	5.33 ± 3.80	4.24 ± 2.24	0.08
Triglycerides (mg/dL)	158.13 ± 82.53	125.66 ± 46.49[a]	0.04
Total cholesterol (mg/dL)	179.33 ± 35.17	181.20 ± 35.89	0.82
HDL-c (mg/dL)	41.06 ± 16.11	43.33 ± 18.28[a]	0.04
LDL-c (mg/dL)	105.40 ± 24.02	106.73 ± 20.78	0.91
VLDL-c (mg/dL)	31.66 ± 16.47	23.93 ± 8.71[a]	0.02
TSH (IU/ml)	1.26 ± 2.990.56	1.47 ± 0.65	0.18

BMI, body mass index; VO_2max, maximum oxygen consumption; 1RM, one maximum repetition; BP, blood pressure; HbA1c, glycated hemoglobin; HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; LDL-c, low-density lipoprotein cholesterol; RMR, resting metabolic rate; T2D, type 2 diabetes mellitus; TSH, thyroid-stimulating hormone; VLDL-c, very-low density lipoprotein cholesterol.

[a]*P < 0.05, t-test dependent samples. Values are in mean ± standard deviation.*

FNDC5/irisin, we observed an increase in the post 60′ moment, when compared to the pre (P = 0.04) and post 30′ (P = 0.03) moments in the first-training session (**Figure 3D**). Natriuretic peptides were increased in the post 30′ (P < 0.01) moment when compared to the pre-moment in the first training session (**Figure 3E**). For meteorin-like, there was a decrease in the post 30′ (P = 0.01) when compared to post moment (**Figure 3F**). No significant changes were observed for FGF21, BMP4, and BMP7 in the first session (**Figures 3G–I**).

The results of the last training session are shown in **Figure 4**. There was an FNDC5/irisin decrease in the post 60′ compared to the post (P < 0.01) and post 30′ (P < 0.01) moments (**Figure 4D**). Natriuretic peptides were increased in the post 60′ moment when compared with the pre (P = 0.01) and post 30′ (P = 0.01) moments (**Figure 4E**). Again there was a decrease in meteorin-like, but this time at the post 60′ moment (P = 0.01) when compared to the post moment (**Figure 4F**). BMP4 exhibited an increase in the post moment when compared to the pre moment (P < 0.01) in the post-training session (**Figure 4H**). No significant changes were observed for IL4, IL13, IL33, FGF21, and BMP7 in the last session (**Figures 4A–C,G,I**).

The differences between first and last time-course sessions (sessions × time and AUC) are shown in **Figure 5**. IL4 (P = 0.04) (**Figure 5A**) and Natriuretic peptides (P = 0.01)

TABLE 3 | Serum thermogenesis inductors in the fasting and fed state before training.

	Pre fast	Post fast	Pre fed	Post fed
IL4 (pg/ml)	2.68 ± 1.91*	2.71 ± 1.71$^{\#}$	4.61 ± 1.57	4.83 ± 1.22
IL13 (pg/ml)	72.35 ± 16.67	63.85 ± 15.01$^{\#}$	505.18 ± 821.45	515.44 ± 756.83
IL33 (pg/ml)	10.86 ± 5.14*	12.17 ± 5.17$^{a\#\&}$	15.97 ± 9.33	14.32 ± 8.53
BMP4 (pg/ml)	19.59 ± 19.45	25.40 ± 28.24	22.08 ± 13.93	21.81 ± 9.06
BMP7 (pg/ml)	22.57 ± 21.83	21.88 ± 17.02	17.72 ± 18.56	18.05 ± 24.67
FGF21 (pg/ml)	145.32 ± 170.36	139.25 ± 172.59	187.70 ± 230.38	169.80 ± 198.09
NAT. PEP. (ng/ml)	3.23 ± 3.42*	3.83 ± 4.27$^{\#}$	0.27 ± 0.12	0.23 ± 0.11
Meteorin-like (pg/ml)	330.84 ± 77.29*	329.54 ± 80.16$^{\#}$	90.34 ± 59.88	94.62 ± 47.57
FNDC5/irisin (μg/ml)	1.30 ± 0.64*	1.63 ± 0.71$^{a\#}$	0.80 ± 0.66	1.19 ± 1.10

IL, interleukin; BMP, bone morphogenic protein; FGF, fibroblast growth factor; NAT. PEP., natriuretic peptides.
$^{a}P < 0.05$, dependent t-test pre versus post.
$^{*}P < 0.05$, dependent t-test fed versus fasting state in pre-training.
$^{\#}P < 0.05$, dependent t-test, fed versus fasting state in post-training.
$^{\&}P \leq 0.05$, ANCOVA, difference pre–post response between fasting and feeding. n = 15, except FNDC5/irisin n = 8 and NAT. PEP. n = 12 in fed. Values are in mean ± standard deviation.

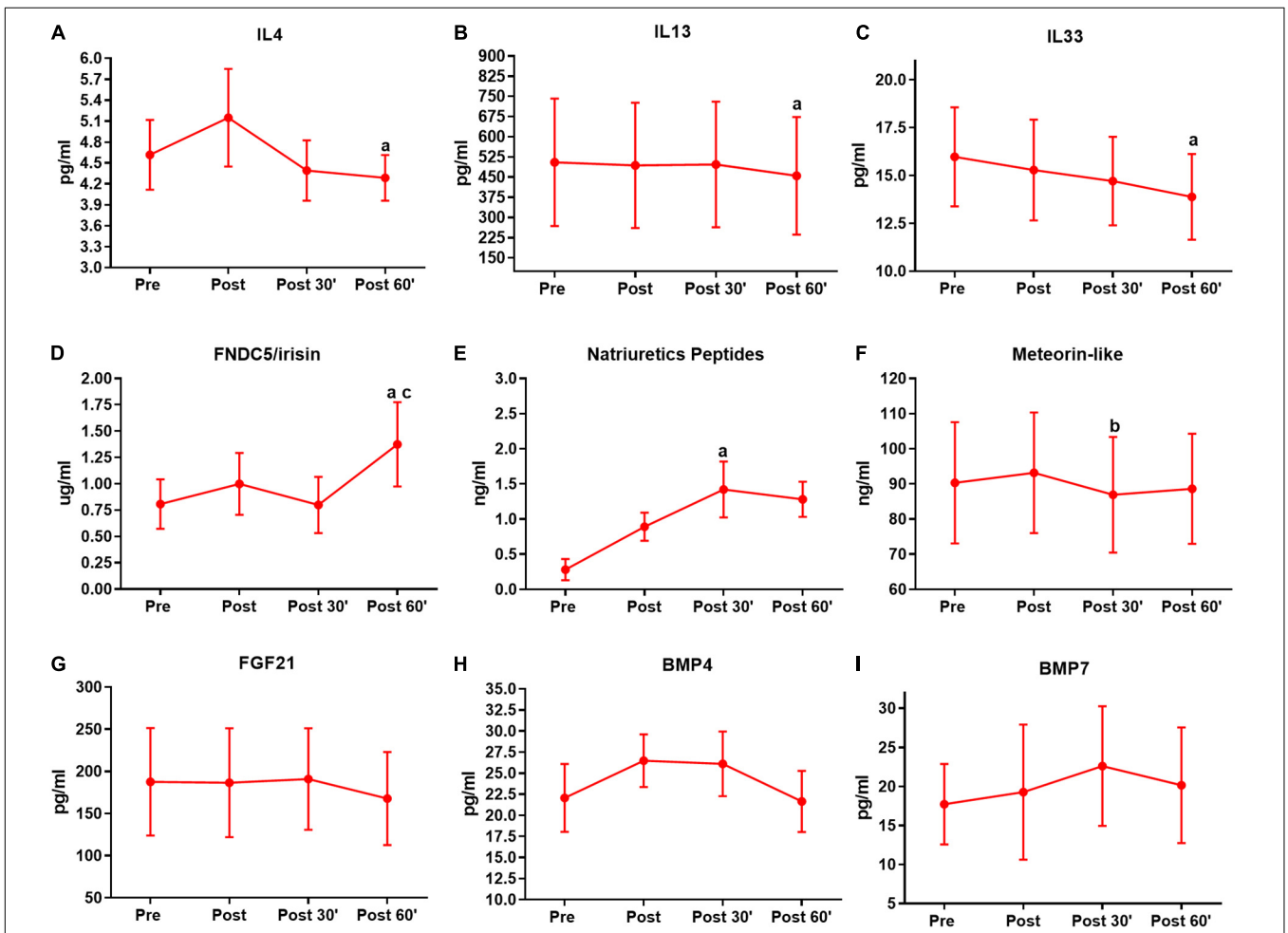

FIGURE 3 | Serum pro-thermogenic/anti-inflammatory inductors time-course in the first training session. **(A)** Interleukin 4 (IL4). **(B)** IL 13. **(C)** IL33. **(D)** FNDC5/irisin. **(E)** Natriuretic peptides. **(F)** Meteorin-like. **(G)** Fibroblast growth factor 21 (FGF21). **(H)** Bone morphogenic protein 4 (BMP4). **(I)** BMP7. $^{a}P < 0.05$, difference from the moment pre. $^{b}P < 0.05$, difference form the moment post. $^{c}P < 0.05$, difference from the moment 30′. $n = 15$ per group, except FNDC5/irisin $n = 8$; and natriuretic peptides $n = 12$, both in feeding group. Values are in mean ± standard error.

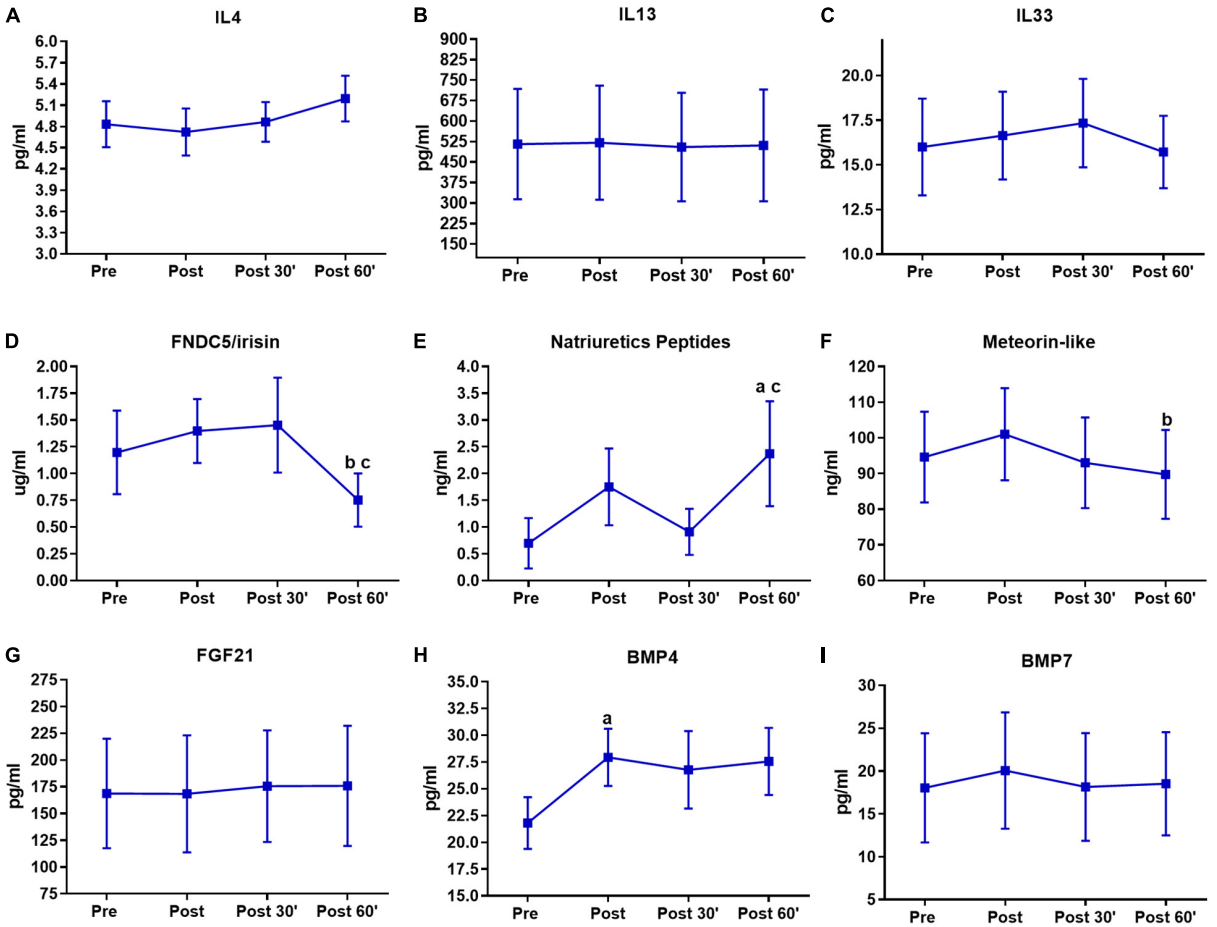

FIGURE 4 | Serum pro-thermogenic/anti-inflammatory inductors time-course in the last training session. **(A)** IL4. **(B)** IL 13. **(C)** IL33. **(D)** FNDC5/irisin. **(E)** Natriuretic peptides. **(F)** Meteorin-like. **(G)** FGF21. **(H)** BMP4. **(I)** BMP7. $^{a}P < 0.05$, difference from the moment pre. $^{b}P < 0.05$, difference form the moment post. $^{c}P < 0.05$, difference from the moment 30′. $n = 15$ per group, except FNDC5/irisin $n = 8$; and natriuretic peptides $n = 12$, both in feeding group. Values are in mean ± standard error.

(**Figure 5E**) presented differences in post 60′ moment between sessions, while this difference was observed for IL33 in post 30′ moment ($P = 0.04$) (**Figure 5C**). Natriuretic peptides showed also difference in last session post 60′ to moments pre ($P = 0.01$) and post ($P = 0.01$) of pre-training session (**Figure 5E**). Different AUCs were observed between sessions in FNDC5/irisin, natriuretic peptides, and meteorin-like (**Figures 5D–F**). No significant changes were observed for IL4, IL13, IL33, FGF21, BMP4, and BMP7 between the first and last sessions (**Figures 5A–C,G–I**).

DISCUSSION

In the present study, we evaluated the chronic and acute training responses and the fed/fasted state of serum pro-thermogenic/anti-inflammatory inducers in overweight type 2 diabetics individuals. We show that adaptation to physical training and a better metabolic pattern favor an improvement in the acute secretory pattern in part of the pro-thermogenic and anti-inflammatory substances. In pre- and post-training, fed and fasting states also interfere differently in these substances, where fasting interferes in the increase of myokines, while the fed state induces an increase in interleukins.

The clinical outcomes support that the other results of the present study after the training period occurred simultaneously with a better metabolic pattern obtained by the CT practice. The CT here applied induced the well-known benefits of strength training (increase in strength and muscle mass) and AT (improvement of aerobic capacity and lipid metabolism, as well as a decrease in body fat and resting heart rate) (Colberg et al., 2010, 2016). In addition, our group observed additional improvements using CT when compared to the strength and AT alone (Libardi et al., 2012), decreased low-grade subclinical inflammation and insulin resistance (Brunelli et al., 2015; Bonfante et al., 2017), interferes in concentrations of certain myokines (Brunelli et al., 2015; Bonfante et al., 2017), and improve global metabolome profile interfering in metabolic pathways related to glucose metabolism, insulin signaling and catecholamines biosynthesis (Duft et al., 2017). Both aerobic and strength training may

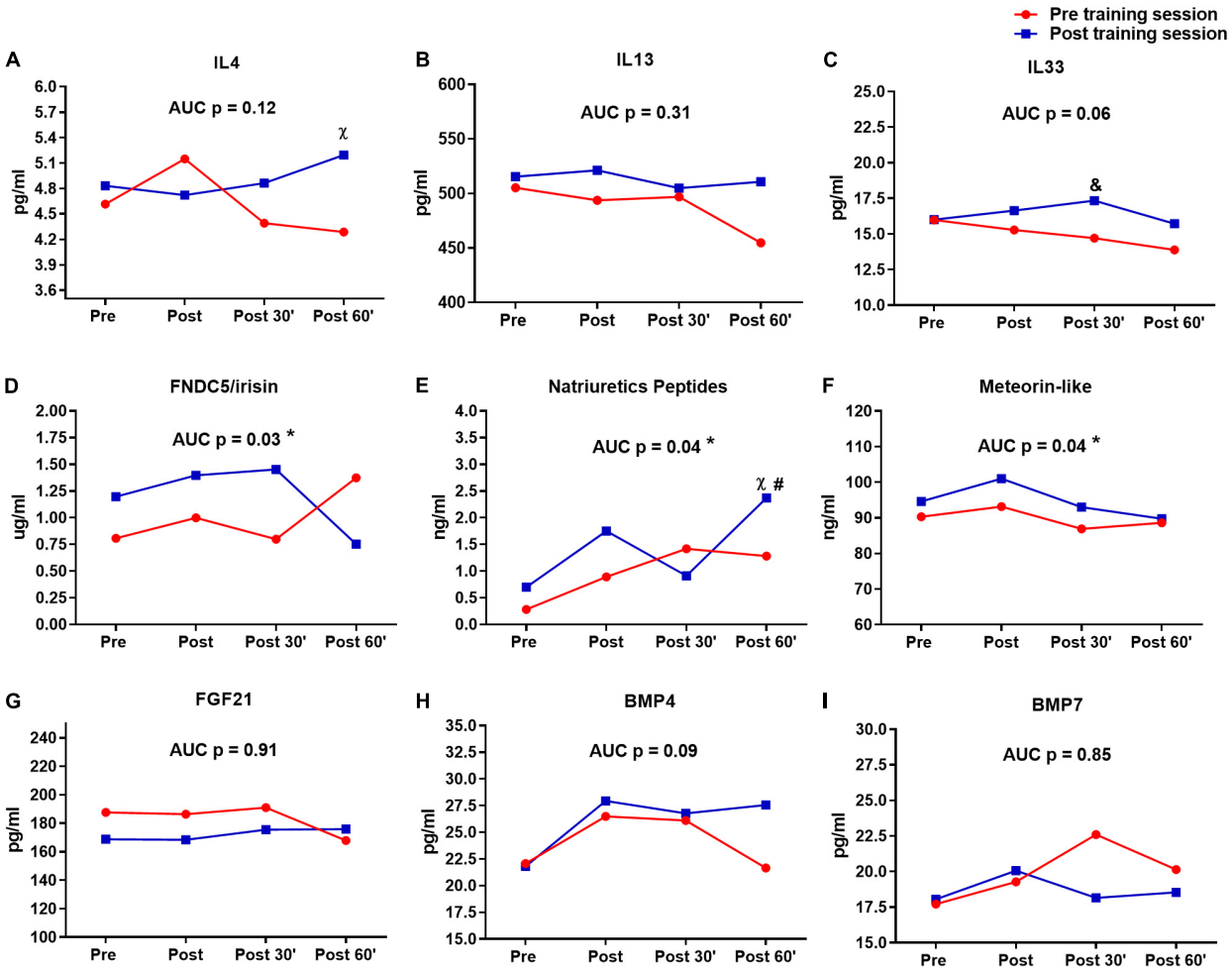

FIGURE 5 | Serum pro-thermogenic/anti-inflammatory inductors time-course in the pre- and post-training sessions. **(A)** IL4. **(B)** IL 13. **(C)** IL33. **(D)** FNDC5/irisin. **(E)** Natriuretic peptides. **(F)** Meteorin-like. **(G–I)** BMP7. $^{\&}P < 0.05$ (Tukey *post hoc* after group × time interaction in ANOVA), difference between sessions in moment 30′. $^{\chi}P < 0.05$ (Tukey *post hoc* after group × time interaction in ANOVA), difference between sessions in moment 60′. $^{\#}P < 0.05$ (Tukey *post hoc* after group × time interaction in ANOVA), difference to moments pre and post of pre training session. $^{*}P < 0.05$ (dependent *t*-test), difference between sessions in area under curve (AUC). $n = 15$ per group, except FNDC5/irisin $n = 8$; and natriuretic peptides $n = 12$, both in feeding group. Values are in mean.

have influenced the improvement of glycemic metabolism since there is a decrease in glycemic levels and glycated hemoglobin. But, for insulin resistance, only a decreasing trend is observed ($P = 0.08$). This result may have been influenced by the method of analysis (HOMA-IR) or the number of subjects analyzed. Although the HOMA-IR method is widely used to analyze insulin resistance, the gold standard method to evaluate this purpose is the hyperinsulinemic–euglycemic clamp (Kim, 2009). Perhaps, if the clamp method had been used, we could observe insulin resistance results that corroborate with other glycemic metabolism markers here evaluated.

The training effects on the concentrations of IL-33 and FNDC5/irisin can be considered positive due to the metabolic benefits and anti-inflammatory effects of these two substances (Bostrom et al., 2012; Brestoff et al., 2015). Curiously, these differences between pre- and post-training seen in IL33 and FNDC5/irisin occurred only on fasting. This is probably due to the complex interaction between metabolism, energy substrates, hormones involved in the control of hunger/satiety and energy demand, the immune system, and the individuality of the individuals to respond to all these interactions. In fasting, a lower incidence of these interferences and certain patterns of organ responses are more easily observed (Di Francesco et al., 2018; Wallis and Gonzalez, 2019). In addition, the correlation data give evidence of the beneficial associations between these pro-thermogenic/anti-inflammatory substances with metabolic control observed in the literature (Pedersen and Febbraio, 2012), as well as the CT contribution to the modulation of these factors.

In the acute effects of the training session, the interleukin results show that with adaptation to training the serum concentration of these substances tend to maintain and increase sharply after exercise, which is a relevant aspect due to their pro-thermogenic, anti-inflammatory, myogenic differentiation, and growth, and muscle regeneration (Nguyen et al., 2011;

Possidonio et al., 2011; Rao et al., 2014; Brestoff et al., 2015; Schiaffino et al., 2017). Meteorin-like is a myokine associated with IL4, IL13, certain lymphocytes, decreased inflammation, and improved insulin resistance (Rao et al., 2014; Li et al., 2015; Jung et al., 2018). Although these associations are not baseline observed, there is a post-training elevation in AUC for this myokine associated with a non-decrease of interleukins.

In the three established myokines analyzed (FNDC5/irisin, meteorin-like, and natriuretics peptides) we have in common the increase of AUC in post-training. Although FNDC5/irisin shows a significant increase only in the first training session and meteorin-like does not have a significant increase in any of the sessions, the AUC increase in post-training indicates a better secretory pattern of these substances in the total time-course analyzed. The enhancement of the natriuretic peptides increase is probably due to the fact that the cardiac musculature is the main secretion site of this peptide (Ramos et al., 2015). The beneficial cardiovascular adaptations traditionally provided by CT, such as increased left ventricular ejection volume and, consequently, cardiac output must have influenced this result. This idea is supported by previous findings, which show that cardiac output during exercise is related to the secretion of natriuretic peptides (Yoshiga et al., 2019).

The results of BMP4 also have acute peaks of secretion optimized with adaptation to training. Curiously, other studies should focus on the evaluation of BMP4 as a myokine since it is expressed in musculoskeletal tissues areas that suffer secretive influences from muscle contraction induced by physical exercises and its metabolic/molecular effects (Tarantino et al., 2015).

Together, these results of acute secretion of pro-thermogenic and anti-inflammatory inducers show that secretory peaks after the training session present an important role in providing the benefits of physical training. Besides, training adaptations favor optimization in the acute secretory pattern of these substances. In addition to training adaptations, the type of exercises, as well as the metabolic pattern of the population analyzed, can also influence the secretion of substances induced by metabolism (Petriz et al., 2017). Thus, the metabolic improvement observed over 16 weeks of training, may also have influenced the present results of the post-training session.

The time-course chosen to evaluate these acute responses was based on other studies with myokines and pro-thermogenic substances or energy needs related (Pedersen and Febbraio, 2012; Petriz et al., 2017; Fox et al., 2018), which usually end up occurring during or in the minutes following the practice of physical exercise. The use of CT protocol was chosen because it is the type of activity recommended for health promotion in this population, including individuals with T2D (Colberg et al., 2010, 2016). In addition, both endurance and strength exercise can also interfere in peptides secretory tissues (for example muscle and fat cells) (Roca-Rivada et al., 2013; Eckardt et al., 2014). For FNDC5/irisin, for example, endurance exercise seems to be the main stimulator (Bostrom et al., 2012; Lee et al., 2014), although acute resistance exercise also leads to a transient increase in these peptides (Nygaard et al., 2015). Further studies should focus on analyzing the responses of different types of exercise protocols and other time-courses since other protocols may promote different secretory peaks. For example, protocols with high-intensity interval training induce the EPOC effect (excess post-exercise oxygen consumption) and consequent additional energy demand for several hours after practicing a training session (Maehlum et al., 1986).

Some studies have observed that a caloric meal or the postprandial period are factors that promote increased secretion of pro-thermogenic products (Vosselman et al., 2013; Din et al., 2017). This may be indicative of the reason for the increase of IL4, IL13, and IL33 in the postprandial moment.

However, caloric restriction in animal models increases IL4, IL13, and IL33, noradrenaline, and consequent thermogenesis in order to generate lipolysis and supply the calorie deficit (Fabbiano et al., 2016, 2018). A possible explanation for this divergence of results is the studies with food deprivation in animals present more aggressive restrictive strategies, differently from the present study. Another aspect that may be related to the decrease of these interleukins, is the fact that fasting induces an increase in cortisol, which influences a functional and secretory decrease of immune system components (Nakamura et al., 2016).

The indication of the increase of the thermogenic inducers natriuretic peptides, meteorin-like, and FNDC5/irisin by fasting may be related to thermal adaptations to compensate for the period without food since there is a certain thermal effect provided by food intake (Brown et al., 2015). Curiously, prolonged fasting in animal models also increased serum concentrations of certain myokines, for example, FNDC5/irisin (Roca-Rivada et al., 2013).

All this context involving serum pro-thermogenic/anti-inflammatory inducers by the effects of exercise and fasting/fed is important due to the association of high concentrations with metabolic health. FNDC5/irisin and natriuretic peptides, for example, have exhibited a decrease in T2D subjects when compared to healthy individuals (Du et al., 2016; Verboven et al., 2017). In addition, primary results of the present research project showed type 2 diabetics individuals with smaller IL4, IL13, IL33, meteorin-like and BMP4 levels when compared to healthy individuals (Bonfante et al., in review). Interestingly, these differences are not observed after training for IL4, meteorin-like, and IL4. Thus, non-pharmacological strategies as physical exercise or food behavior to induce higher concentrations of pro-thermogenic/anti-inflammatory are important, since the increased activity of thermogenic and anti-inflammatory tissues, such as brown adipose tissue, is associated with protection against several cardiometabolic diseases (Becher et al., 2021).

The present study has some limitations, such as comparisons between fasted and fed states on different days. However, certain procedures were taken to minimize this, such as standardized meals and times, assessments performed on the same day of the week, and a short period between assessments. The lack of a CG in the baseline chronic outcomes is also a limitation, but this was the main objective of the primary study of the present research project. Importantly, the results of this primary study (randomized and controlled) corroborate the chronic baseline results found in the present study. Although the number of subjects and statistical power had an adequate standard, probably a larger sample of subjects could support that results with a

tendency toward statistical significance (P between 0.05 and 0.08) become significant ($P < 0.05$), especially in the case of the technical limitation, which decreased the number of analyzed samples of FNDC5/irisin and natriuretic peptides. Finally, despite the HOMA-IR method being widely used to analyze insulin resistance, not having used the hyperinsulinemic–euglycemic clamp for this purpose also is a limitation.

In conclusion, adaptation to physical training and a better metabolic pattern promote an improvement in the acute secretory pattern in part of pro-thermogenic and anti-inflammatory substances, which should contribute to the metabolic benefits achieved by incorporating an active lifestyle in overweight individuals with T2D. Baseline analyses showed that only part of these substances indicates an increase (IL33 and FNDC5/irisin), although important correlations were observed between these substances with several analyzed clinical variables.

The fed and fasting states also interfere differently in part of these pro-thermogenic and anti-inflammatory substances, where fasting interferes in the increase of myokines, while the fed state induces an increase in interleukins.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by https://www.avivasysbio.com/. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CC supervised the study. IB, RD, KM, JT, EF, AR, DB, MM, MC-M, LV, and CC performed hypothesis, generation, contributed to the design, data analysis, interpretation of results, and manuscript preparation. IB, RD, KM, JT, EF, AR, and DB conducted the experiments, tests, training sessions, and data analysis. IB was the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors edited and approved the final manuscript.

FUNDING

This study was supported by the São Paulo State Research Support Foundation (FAPESP), São Paulo, Brazil, Regular Research Grants, Process: 2016/08751-3; Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil, Financing Code 001; and National Council for Scientific and Technological Development (CNPQ), Brazil, Financing Code 303571/2018-7.

ACKNOWLEDGMENTS

IB thanks Luiz Carlos Bonfante (*In memoriam*). We thank the participants; Obesity and Comorbidities Research Center, University of Campinas; Faculty of Physical Education, University of Campinas; Integrated Teaching, Research and Extension Laboratory, School of Physical Education, University of Campinas. Finally, we thank the funding agencies, cited mentioned below.

REFERENCES

Abdul-Hameed, U., Rangra, P., Shareef, M. Y., and Hussain, M. E. (2012). Reliability of 1-repetition maximum estimation for upper and lower body muscular strength measurement in untrained middle aged type 2 diabetic patients. *Asian J. Sports Med.* 3, 267–273. doi: 10.5812/asjsm.34549

Becher, T., Palanisamy, S., Kramer, D. J., Eljalby, M., Marx, S. J., Wibmer, A. G., et al. (2021). Brown adipose tissue is associated with cardiometabolic health. *Nat. Med.* 27, 58–65. doi: 10.1038/s41591-020-1126-7

Benedini, S., Codella, R., Caumo, A., Marangoni, F., and Luzi, L. (2011). Different circulating ghrelin responses to isoglucidic snack food in healthy individuals. *Horm. Metab. Res.* 43, 135–140. doi: 10.1055/s-0030-1269900

Benedini, S., Codella, R., Caumo, A., Terruzzi, I., and Luzi, L. (2019). Single bout of low-intensity exercise produces modestly favorable changes in glycemic and lipidemic profiles after ingestion of non-isoglucidic breakfasts. *Nutrition* 58, 57–64. doi: 10.1016/j.nut.2018.06.005

Bonfante, I. L., Chacon-Mikahil, M. P., Brunelli, D. T., Gaspari, A. F., Duft, R. G., Lopes, W. A., et al. (2017). Combined training, FNDC5/irisin levels and metabolic markers in obese men: a randomised controlled trial. *Eur. J. Sport Sci.* 17, 629–637. doi: 10.1080/17461391.2017.1296025

Bostrom, P., Wu, J., Jedrychowski, M. P., Korde, A., Ye, L., and Lo, J. C. (2012). A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 481, 463–468.

Brestoff, J. R., Kim, B. S., Saenz, S. A., Stine, R. R., Monticelli, L. A., Sonnenberg, G. F., et al. (2015). Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. *Nature* 519, 242–246. doi: 10.1038/nature14115

Brown, L., Poudyal, H., and Panchal, S. K. (2015). Functional foods as potential therapeutic options for metabolic syndrome. *Obes. Rev.* 16, 914–941. doi: 10.1111/obr.12313

Brunelli, D. T., Chacon-Mikahil, M. P., Gaspari, A. F., Lopes, W. A., Bonganha, V., and Bonfante, I. L. (2015). Combined training reduces subclinical inflammation in obese middle-age men. *Med. Sci. Sports Exerc.* 47, 2207–2215. doi: 10.1249/MSS.0000000000000658

Colberg, S. R., Albright, A. L., Blissmer, B. J., Braun, B., Chasan-Taber, L., and Fernhall, B. (2010). Exercise and type 2 diabetes: american college of sports medicine and the american diabetes association: joint position statement. exercise and type 2 diabetes. *Med. Sci. Sports Exerc.* 42, 2282–2303.

Colberg, S. R., Sigal, R. J., Yardley, J. E., Riddell, M. C., Dunstan, D. W., and Dempsey, P. C. (2016). Physical activity/exercise and diabetes: a position statement of the American diabetes association. *Diabetes Care* 39, 2065–2079. doi: 10.2337/dc16-1728

Di Francesco, A., Di Germanio, C., Bernier, M., and De Cabo, R. (2018). A time to fast. *Science* 362, 770–775.

Din, M. U., Raiko, J., Saari, T., Saunavaara, V., Kudomi, N., Solin, O., et al. (2017). Human brown fat radiodensity indicates underlying tissue composition and systemic metabolic health. *J. Clin. Endocrinol. Metab.* 102, 2258–2267. doi: 10.1210/jc.2016-2698

Din, M. U., Saari, T., Raiko, J., Kudomi, N., Maurer, S. F., Lahesmaa, M., et al. (2018). Postprandial Oxidative Metabolism of Human Brown Fat Indicates Thermogenesis. *Cell Metab.* 28, 207–216 e203. doi: 10.1016/j.cmet.2018.05.020

Du, X. L., Jiang, W. X., and Lv, Z. T. (2016). Lower circulating irisin level in patients with diabetes mellitus: a systematic review and meta-analysis. *Horm. Metab. Res.* 48, 644–652. doi: 10.1055/s-0042-108730

Duft, R. G., Castro, A., Bonfante, I. L. P., Brunelli, D. T., Chacon-Mikahil, M. P. T., and Cavaglieri, C. R. (2017). Metabolomics approach in the investigation of metabolic changes in obese men after 24 weeks of combined training. *J. Proteome Res.* 16, 2151–2159. doi: 10.1021/acs.jproteome.6b00967

Eckardt, K., Gorgens, S. W., Raschke, S., and Eckel, J. (2014). Myokines in insulin resistance and type 2 diabetes. *Diabetologia* 57, 1087–1099.

Fabbiano, S., Suarez-Zamorano, N., Chevalier, C., Lazarevic, V., Kieser, S., and Rigo, D. (2018). Functional gut microbiota remodeling contributes to the caloric restriction-induced metabolic improvements. *Cell Metab.* 28, 907–921.e907. doi: 10.1016/j.cmet.2018.08.005

Fabbiano, S., Suarez-Zamorano, N., Rigo, D., Veyrat-Durebex, C., Stevanovic Dokic, A., Colin, D. J., et al. (2016). Caloric restriction leads to browning of white adipose tissue through Type 2 immune signaling. *Cell Metab.* 24, 434–446. doi: 10.1016/j.cmet.2016.07.023

Faelli, E., Bisio, A., Codella, R., Ferrando, V., Perasso, L., Panasci, M., et al. (2020). Acute and Chronic Catabolic Responses to CrossFit((R)) and resistance training in young males. *Int. J. Environ. Res. Public Health* 17:7172. doi: 10.3390/ijerph17197172

Fox, J., Rioux, B. V., Goulet, E. D. B., Johanssen, N. M., Swift, D. L., Bouchard, D. R., et al. (2018). Effect of an acute exercise bout on immediate post-exercise irisin concentration in adults: a meta-analysis. *Scand. J. Med. Sci. Sports* 28, 16–28. doi: 10.1111/sms.12904

Gleeson, M., Bishop, N. C., Stensel, D. J., Lindley, M. R., Mastana, S. S., and Nimmo, M. A. (2011). The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat. Rev. Immunol.* 11, 607–615. doi: 10.1038/nri3041

Gomez-Ambrosi, J., Silva, C., Galofre, J. C., Escalada, J., Santos, S., Gil, M. J., et al. (2011). Body adiposity and type 2 diabetes: increased risk with a high body fat percentage even having a normal BMI. *Obesity* 19, 1439–1444. doi: 10.1038/oby.2011.36

Hoffmann, J. M., Grunberg, J. R., Church, C., Elias, I., Palsdottir, V., and Jansson, J. O. (2017). BMP4 gene therapy in mature mice reduces BAT activation but protects from obesity by browning subcutaneous adipose tissue. *Cell Rep.* 20, 1038–1049. doi: 10.1016/j.celrep.2017.07.020

Huh, J. Y., Mougios, V., Kabasakalis, A., Fatouros, I., Siopi, A., Douroudos, et al. (2014). Exercise-induced irisin secretion is independent of age or fitness level and increased irisin may directly modulate muscle metabolism through AMPK activation. *J. Clin. Endocrinol. Metab.* 99, E2154–E2161. doi: 10.1210/jc.2014-1437

Huh, J. Y., Panagiotou, G., Mougios, V., Brinkoetter, M., Vamvini, M. T., Schneider, B. E., et al. (2012). FNDC5 and irisin in humans: I. predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. *Metabolism* 61, 1725–1738. doi: 10.1016/j.metabol.2012.09.002

Jung, T. W., Lee, S. H., Kim, H. C., Bang, J. S., Abd El-Aty, A. M., and Hacimuftuoglu, A. (2018). METRNL attenuates lipid-induced inflammation and insulin resistance via AMPK or PPARdelta-dependent pathways in skeletal muscle of mice. *Exp. Mol. Med.* 50, 1–11. doi: 10.1038/s12276-018-0147-5

Kajimura, S., and Saito, M. (2014). A new era in brown adipose tissue biology: molecular control of brown fat development and energy homeostasis. *Annu. Rev. Physiol.* 76, 225–249. doi: 10.1146/annurev-physiol-021113-170252

Kim, J. K. (2009). Hyperinsulinemic-euglycemic clamp to assess insulin sensitivity in vivo. *Methods Mol. Biol.* 560, 221–238. doi: 10.1007/978-1-59745-448-3_15

Lee, P., Linderman, J. D., Smith, S., Brychta, R. J., Wang, J., and Idelson, C. (2014). Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. *Cell Metab.* 19, 302–309. doi: 10.1016/j.cmet.2013.12.017

Li, Z. Y., Song, J., Zheng, S. L., Fan, M. B., Guan, Y. F., Qu, Y., et al. (2015). Adipocyte metrnl antagonizes insulin resistance through PPARgamma signaling. *Diabetes Metab. Res. Rev.* 64, 4011–4022. doi: 10.2337/db15-0274

Libardi, C. A., De Souza, G. V., Cavaglieri, C. R., Madruga, V. A., and Chacon-Mikahil, M. P. (2012). Effect of resistance, endurance, and concurrent training on TNF-alpha, IL-6, and CRP. *Med. Sci. Sports Exerc.* 44, 50–56. doi: 10.1249/MSS.0b013e318229d2e9

Lucotti, P., Monti, L. D., Setola, E., Galluccio, E., Gatti, R., Bosi, E., et al. (2011). Aerobic and resistance training effects compared to aerobic training alone in obese type 2 diabetic patients on diet treatment. *Diabetes Res. Clin. Pract.* 94, 395–403. doi: 10.1016/j.diabres.2011.08.002

Maehlum, S., Grandmontagne, M., Newsholme, E. A., and Sejersted, O. M. (1986). Magnitude and duration of excess postexercise oxygen consumption in healthy young subjects. *Metabolism* 35, 425–429. doi: 10.1016/0026-0495(86)90132-0

Modica, S., Straub, L. G., Balaz, M., Sun, W., Varga, L., Stefanicka, P., et al. (2016). Bmp4 promotes a brown to white-like adipocyte shift. *Cell Rep.* 16, 2243–2258. doi: 10.1016/j.celrep.2016.07.048

Nakamura, Y., Walker, B. R., and Ikuta, T. (2016). Systematic review and meta-analysis reveals acutely elevated plasma cortisol following fasting but not less severe calorie restriction. *Stress* 19, 151–157. doi: 10.3109/10253890.2015.1121984

Nguyen, K. D., Qiu, Y., Cui, X., Goh, Y. P., Mwangi, J., David, T., et al. (2011). Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. *Nature* 480, 104–108. doi: 10.1038/nature10653

Nygaard, H., Slettalokken, G., Vegge, G., Hollan, I., Whist, J. E., Strand, T., et al. (2015). Irisin in blood increases transiently after single sessions of intense endurance exercise and heavy strength training. *PLoS One* 10:e0121367. doi: 10.1371/journal.pone.0121367

Nyssen, S. M., Santos, J. G., Barusso, M. S., Oliveira, A. D. Jr., Lorenzo, V. A., et al. (2013). Levels of physical activity and predictors of mortality in COPD. *J. Bras. Pneumol.* 39, 659–666. doi: 10.1590/S1806-37132013000600004

Otero-Diaz, B., Rodriguez-Flores, M., Sanchez-Munoz, V., Monraz-Preciado, F., Ordonez-Ortega, S., and Becerril-Elias, V. (2018). Exercise induces white adipose tissue browning across the weight spectrum in humans. *Front. Physiol.* 9:1781. doi: 10.3389/fphys.2018.01781

Pedersen, B. K., and Febbraio, M. A. (2012). Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nat. Rev. Endocrinol.* 8, 457–465. doi: 10.1038/nrendo.2012.49

Petriz, B. A., Gomes, C. P., Almeida, J. A., De Oliveira, G. P. Jr., Ribeiro, F. M., et al. (2017). The effects of acute and chronic exercise on skeletal muscle proteome. *J. Cell. Physiol.* 232, 257–269. doi: 10.1002/jcp.25477

Possidonio, A. C., Senna, M. L., Portilho, D. M., Pontes Soares, C., Da Silva, Sampaio, L., et al. (2011). alpha-Cyclodextrin enhances myoblast fusion and muscle differentiation by the release of IL-4. *Cytokine* 55, 280–287. doi: 10.1016/j.cyto.2011.04.018

Qian, S. W., Tang, Y., Li, X., Liu, Y., Zhang, Y. Y., and Huang, H. Y. (2013). BMP4-mediated brown fat-like changes in white adipose tissue alter glucose and energy homeostasis. *Proc. Natl. Acad. Sci. U.S.A.* 110, E798–E807. doi: 10.1073/pnas.1215236110

Ramos, H. R., Birkenfeld, A. L., and De Bold, A. J. (2015). INTERACTING DISCIPLINES: cardiac natriuretic peptides and obesity: perspectives from an endocrinologist and a cardiologist. *Endocr. Connect.* 4, R25–R36. doi: 10.1530/EC-15-0018

Rao, R. R., Long, J. Z., White, J. P., Svensson, K. J., Lou, J., and Lokurkar, I. (2014). Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. *Cell* 157, 1279–1291. doi: 10.1016/j.cell.2014.03.065

Roca-Rivada, A., Castelao, C., Senin, L. L., Landrove, M. O., Baltar, J., Belen Crujeiras, A., et al. (2013). FNDC5/irisin is not only a myokine but also an adipokine. *PLoS One* 8:e60563. doi: 10.1371/journal.pone.0060563

Schiaffino, S., Pereira, M. G., Ciciliot, S., and Rovere-Querini, P. (2017). Regulatory T cells and skeletal muscle regeneration. *FEBS J.* 284, 517–524. doi: 10.1111/febs.13827

Schulz, T. J., Huang, P., Huang, T. L., Xue, R., Mcdougall, L. E., Townsend, K. L., et al. (2013). Brown-fat paucity due to impaired BMP signalling induces compensatory browning of white fat. *Nature* 495, 379–383. doi: 10.1038/nature11943

Tarantino, U., Scimeca, M., Piccirilli, E., Tancredi, V., Baldi, J., Gasbarra, E., et al. (2015). Sarcopenia: a histological and immunohistochemical study on age-related muscle impairment. *Aging Clin. Exp. Res.* 27, S51–S60. doi: 10.1007/s40520-015-0427-z

Tseng, Y. H., Kokkotou, E., Schulz, T. J., Huang, T. L., Winnay, J. N., and Taniguchi, C. M. (2008). New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454, 1000–1004. doi: 10.1038/nature07221

Verboven, K., Hansen, D., Jocken, J. W. E., and Blaak, E. E. (2017). Natriuretic peptides in the control of lipid metabolism and insulin sensitivity. *Obes. Rev.* 18, 1243–1259. doi: 10.1111/obr.12598

Villarroya, F., and Vidal-Puig, A. (2013). Beyond the sympathetic tone: the new brown fat activators. *Cell Metab.* 17, 638–643. doi: 10.1016/j.cmet.2013.02.020

Vosselman, M. J., Brans, B., Van Der Lans, A. A., Wierts, R., Van Baak, M. A., Mottaghy, F. M., et al. (2013). Brown adipose tissue activity after a high-calorie meal in humans. *Am. J. Clin. Nutr.* 98, 57–64. doi: 10.3945/ajcn.113.059022

Wallis, G. A., and Gonzalez, J. T. (2019). Is exercise best served on an empty stomach? *Proc. Nutr. Soc.* 78, 110–117. doi: 10.1017/S0029665118002574

Yoshiga, C., Dawson, E. A., Volianitis, S., Warberg, J., and Secher, N. H. (2019). Cardiac output during exercise is related to plasma atrial natriuretic peptide but not to central venous pressure in humans. *Exp. Physiol.* 104, 379–384. doi: 10.1113/EP087522

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frontiers in Physiology

REVIEW
published: 15 February 2022
doi: 10.3389/fphys.2022.808374

The Functions and Mechanisms of Low-Level Laser Therapy in Tendon Repair (Review)

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Tendon injury is a common disease of the musculoskeletal system, accounting for roughly 30%–40% of sports system disorder injuries. In recent years, its incidence is increasing. Many studies have shown that low-level laser therapy (LLLT) has a significant effect on tendon repair by firstly activating cytochrome C oxidase and thus carrying out the photon absorption process, secondly acting in all the three phases of tendon repair, and finally improving tendon recovery. The repair mechanisms of LLLT are different in the three phases of tendon repair. In the inflammatory phase, LLLT mainly activates a large number of VEGF and promotes angiogenesis under hypoxia. During the proliferation phase, LLLT increases the amount of collagen type III by promoting the proliferation of fibroblasts. Throughout the remodeling phase, LLLT mainly activates M2 macrophages and downregulates inflammatory factors, thus reducing inflammatory responses. However, it should also be noted that in the final phase of tendon repair, the use of LLLT causes excessive upregulation of some growth factors, which will lead to tendon fibrosis. In summary, we need to further investigate the functions and mechanisms of LLLT in the treatment of tendon injury and to clarify the nature of LLLT for the treatment of diverse tendon injury diseases.

Keywords: low-level laser therapy, tendon repair, tendinopathy, mechanism, function

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *03 November 2021*
Accepted: *24 January 2022*
Published: *15 February 2022*

Citation:
Lyu K, Liu X, Jiang L, Chen Y, Lu J, Zhu B, Liu X, Li Y, Wang D and Li S (2022) The Functions and Mechanisms of Low-Level Laser Therapy in Tendon Repair (Review). Front. Physiol. 13:808374. doi: 10.3389/fphys.2022.808374

INTRODUCTION

In recent years, researchers report that the prevalence of tendon injuries continues to rise, with young people being the most vulnerable group, and rotator cuff muscles and the Achilles tendon being the most common sites of injury (Xu and Murrell, 2008; Thomopoulos et al., 2015; Genc et al., 2020). It is generally thought that the primary cause of tendon injury is overuse, which not only alters the tendon structure but also causes many negative reactions such as tendon swelling, irregular collagen arrangement, and an increase in pathological molecules (Xu and Murrell, 2008; Kaux et al., 2011; Sereysky et al., 2013; Aicale et al., 2018).

Currently, tendon injuries are treated with a comprehensive range of treatments, which include conservative treatments (such as ultrasound, shock wave, platelet rich plasma, and low-level laser therapy, LLLT), surgery, and specific exercises to help with rehabilitation. Over the past decade, LLLT use has been increasingly examined in many clinical studies. It has

been used to treat tendon injuries, with excellent results in tendon repair (Sereysky et al., 2013; Lipman et al., 2018). LLLT, also known as photobiomodulation, primarily reduces the degree of tendon injury by topical application of short-wavelength monochromatic light. The mechanisms of LLLT are mainly related to cytochrome C oxidase, and its functions include the promotion of angiogenesis, the acceleration of cell proliferation, the promotion of metabolism, and the release of inflammatory factors (Nogueira Junior and Moura Junior, 2015; Wang et al., 2016).

Most recent studies have been able to explain the overall mechanisms of LLLT in the treatment of tendon lesions and its treatment advantages. However, because LLLT's mechanisms involve a large number of biomolecular changes and the interactions between them, there are still some unclear points in the study of its therapeutic mechanisms (Andarawis-Puri et al., 2015; Lopes Silva et al., 2020). Consequently, considering the significant impact of LLLT, this review will analyze the various effects of LLLT on tendon repair, explain its mechanisms of action in different stages of repair, and then present a discussion of its potential therapeutic use going forward. The overall aim of this review is to provide evidence for the future treatment of tendon lesions with LLLT and to explain more fully the explicit role of LLLT in the healing process.

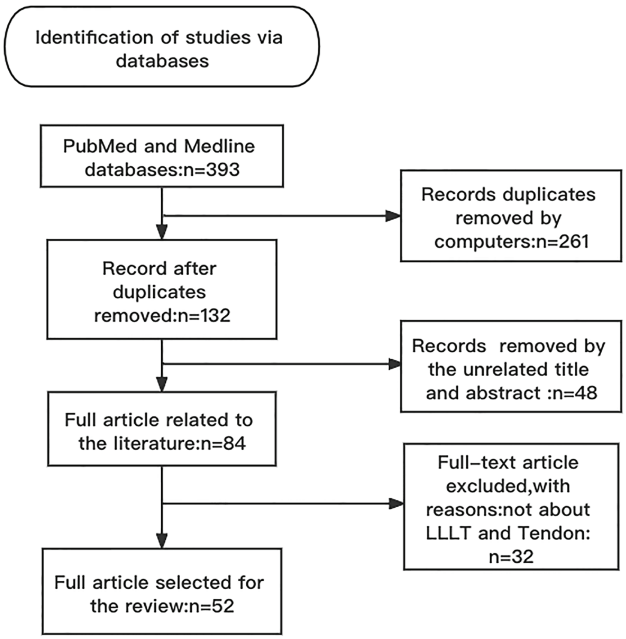

FIGURE 1 | Article retrieval flow chart with inclusion and exclusion process.

Search Strategy

(i) Search site: articles are from PubMed, a database of papers on biomedical science. (ii) Database: MEDLINE. (iii) Keywords: LLLT, tendon repair, tendinopathy, mechanism. (iv) Boolean algorithm: (LLLT OR photobiomodulation) AND (Tendinopathy OR Tendon injuries OR Tendon repair) AND function AND mechanism. (v) Retrieving timeframe: we searched the selected journals published from 2000 to 2021. (vi) Inclusion and exclusion criteria: articles were included if the topic is related to LLLT and tendon repair, while the article type was a review or an experimental paper. The retrieval process is shown in **Figure 1**.

CHARACTERISTICS AND MERITS OF LLLT FOR TENDON INJURIES IN CLINICAL PRACTICE

There are multiple options for treating tendinopathy that can be divided into surgical and non-surgical treatments. Tendon injuries that warrant operative treatments can be of two types. Firstly, for more severe tendon injuries, surgical therapies as described below are frequently performed, for instance, open debridement, tendonectomy, and tendon grafting, which allow for the removal of degenerative tissue in such a way as to stimulate tendon healing (Andarawis-Puri et al., 2015; Li and Hua, 2016). Secondly, a few tendon impairments that do not respond well to conservative treatment may still necessitate a surgical intervention (Alfredson and Cook, 2007).

The tendon disorders that demand non-operative remedies can also be illustrated in two categories. Primarily, for less symptomatic injuries, we commonly apply conservative treatments such as proper rest, Non-steroidal anti-inflammatory drugs (NSAIDs), injections, cryotherapy, physical therapy, etc. The typical modalities are as follows. NSAIDs have been widely used in clinical practice, but due to their innumerable side effects and lack of therapeutic efficacy, they are no longer used as a preferred treatment modality (Andres and Murrell, 2008). At present, the most respected method in non-surgical treatment is injection therapy, which includes platelet-rich plasma injections, mesenchymal stem cells (MSCs), hyaluronic acid injections, or other injectable therapies. Numerous studies have now proven the superiorities of using MSCs in tendon healing and perhaps in broader applications (Ragni et al., 2021). With numerous origins of MSCs, adipose-derived mesenchymal cells (ASCs) are optimal for inclusion in non-surgical protocols since they promote tendon repair more effectively than bone marrow MSCs (BMSCS). Firstly, ASCs are conveniently isolated from adipose tissue and are less susceptible to destruction. Secondly, they downregulate relevant inflammatory factors, enhance fibroblast proliferation, accelerate tendon cell differentiation, and boost angiogenesis. Finally, they further spur tissue regeneration and boost the process of tendon repair (Shen et al., 2020; Piccionello et al., 2021). While MSCs have considerable benefits in musculoskeletal disorders, there is still a certain risk of infection during the healing process. The effectiveness of MSCs have mostly been verified through animal studies; hence, we still require a wealth of *in vivo* trials to demonstrate the effectiveness of ASCs therapy (Sandona et al., 2021).

Next, postoperative rehabilitation to restore tendon function will be vital, and thus we will rely on certain nonoperative measures to assist in the rehabilitation process. For problems

that will arise during postoperative rehabilitation (such as joint stiffness and muscle atrophy), physical therapy and massage can be utilized in conjunction with centrifugal exercises for training. Physical therapies that have similarities to LLLT include extracorporeal shock wave therapy, ultrasound therapy, and others. Among which, ESWT works by delivering energy deep into the tendon tissue. Studies have shown that ESWT in combination with other therapies (eccentric exercise) can substantially reduce the degree of injury (Stania et al., 2019). On the whole, there are several options for addressing tendon injuries, paradoxically, extensive experiments are still warranted to prove its availability (including *in vitro* and *in vivo* tests). Different treatments for tendon injuries are shown in **Figure 2**.

LLLT has been more widely used in clinical practice. Generally, the effect of LLLT varies with the duration of laser exposure, which invariably involves the biological effects of photobiomodulation (Andres and Murrell, 2008; Fortuna et al., 2018). However, depending on the duration of laser irradiation, its effects on the deleterious sites keep diverse. The short-term effect is that an irradiation lasting a few seconds or minutes will upregulate the ATP content (Clijsen et al., 2017). The long-term effect is that the tendon repair process will be in a phase of cell proliferation after prolonged laser exposure, which accelerates the proliferation of fibroblasts and then promotes the synthesis of collagen type I and III (Fortuna et al., 2018). In clinical practice, nevertheless, there are still no specific treatment parameters or treatment protocols for the diverse array of tendon injuries (Bjordal et al., 2006). Much of the research has examined that when the damaged area receives near-infrared light with a length of 600–1,000 nm and a radiation value of 3–10 J/cm^2, it normally promotes tendon repair to a large extent. In the case of tendon injuries requiring operative therapy, the use of LLLT is typically placed after surgical treatment. First of all, a slice of open tendon injuries are often treated with a laser within 4 J/cm^2, while in contrast, for quite a few closed injuries (degenerative lesions), a laser in the range of 10–50 J/cm^2 should be used (Zein et al., 2018).

THE BASIS OF HOW LLLT WORKS: C CYTCHROME C OXIDASE

Since the low-level laser treatment process is a photochemical reaction, it is closely related to the absorption of photons, which are associated with cytochrome C oxidase (CCO). LLLT will improve ATP synthesis and respiration rate through this process, thus promoting tendon injury repair. Conversely, laser therapy does not work when CCO activation fails (Wang et al., 2016). CCO, as an enzyme at the end of the mitochondrial respiratory chain, mainly performs electron transfer in the process of energy metabolism and promotes REDOX reaction under the condition of cellular hypoxia. Under normal conditions, CCO not only oxidizes the four reduced cytochrome C molecules but also produces four protons, which combine with oxygen to form water and activate ATPase to produce large amounts of ATP. Nitric oxide (NO) can bind to CuB to inhibit this process (Farivar et al., 2014). However, when the injured tissue is irradiated by laser, LLLT can considerably improve CCO

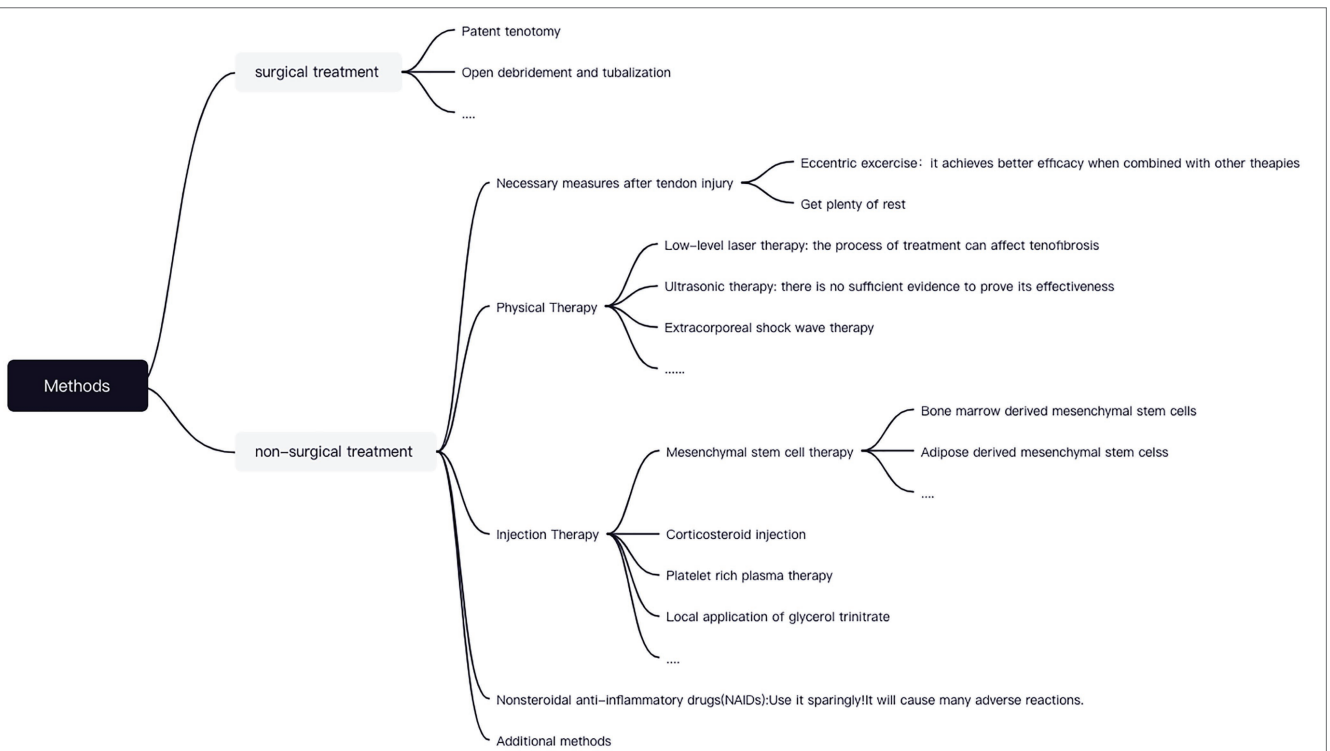

FIGURE 2 | Different treatments for tendon injuries.

activity and enhance the oxidative metabolism of cells (Farivar et al., 2014; Clijsen et al., 2017; Tsai and Hamblin, 2017; Hamblin, 2018), NO can be dissociated, so the respiration rate will increase, and a large amount of ATP will be generated. Consequently, to maintain a balance between oxygen intake and demand, cellular metabolism is improved, and the hemodynamic changes, thus promoting healing of tendons (Farivar et al., 2014; Wang et al., 2016; Tsai and Hamblin, 2017). The absorption of photons is illustrated in **Figure 3**.

MECHANISMS OF ACTION OF LLLT IN THE THREE PHASES OF TENDON REPAIR

The process of tendon healing can be divided into three main phases: the inflammatory phase, the cell proliferation phase, and the tendon shaping phase. The inflammatory phase occurs within 48 h of tendon injury—first, blood clots fill the injured tissue, and then fibrin continues to attach to the damaged tissue as inflammation develops (Lopes Silva et al., 2020). The proliferative phase is mainly characterized by the formation of large amounts of granulation tissue, including the proliferation of fibroblasts and the synthesis of type III collagen. The remodeling phase is characterized by a remodeling of the ECM, which is not only accompanied by a significant reduction or apoptosis of cells but also by a reduction of type III collagen and the promotion of type I collagen synthesis (Andarawis-Puri et al., 2015). The mechanisms of LLLT in the treatment of tendon injury are shown in **Figure 4**. The factors associated with ECM remodeling are shown in **Table 1**.

LLLT Promotes Angiogenesis During the Inflammatory Phase

During the inflammatory phase of tendon repair, LLLT primarily promotes angiogenesis. In general, tendon injury is associated with a series of pathological changes, such as vascular infiltration and upregulation of vascular endothelial growth factors (VEGF; Xu and Murrell, 2008). The mechanisms of LLLT in the treatment of tendon injury are mainly mediated by photobiological stimulation rather than by thermal effect (Rand et al., 2007; Lin et al., 2010). Firstly, the necessary condition for LLLT to promote angiogenesis is hypoxia, and secondly, LLLT can regulate the activity of angiogenic factors. Angiogenesis is mainly associated with hypoxia inducible factor 1α (HIF-1α) activation, large expression of VEGF, and downregulation of matrix metallopeptidase 2 (MMP-2; Hamblin, 2018).

The hypoxia-inducible factor-HIF-1α plays an important role in the process of tendon tissue facing hypoxia because hypoxia is a necessary condition for the formation of new blood vessels. Since the absorption of photons is accompanied by an increase in respiration rate, the amount of oxygen in the tissue drops sharply, thereby activating HIF-1α (Hamblin, 2018).

Hypoxia causes an increase in the number of growth factors. The factor most associated with angiogenesis is vascular endothelial growth factor (VEGF), which has many effects on vascular endothelial, including promoting the establishment of

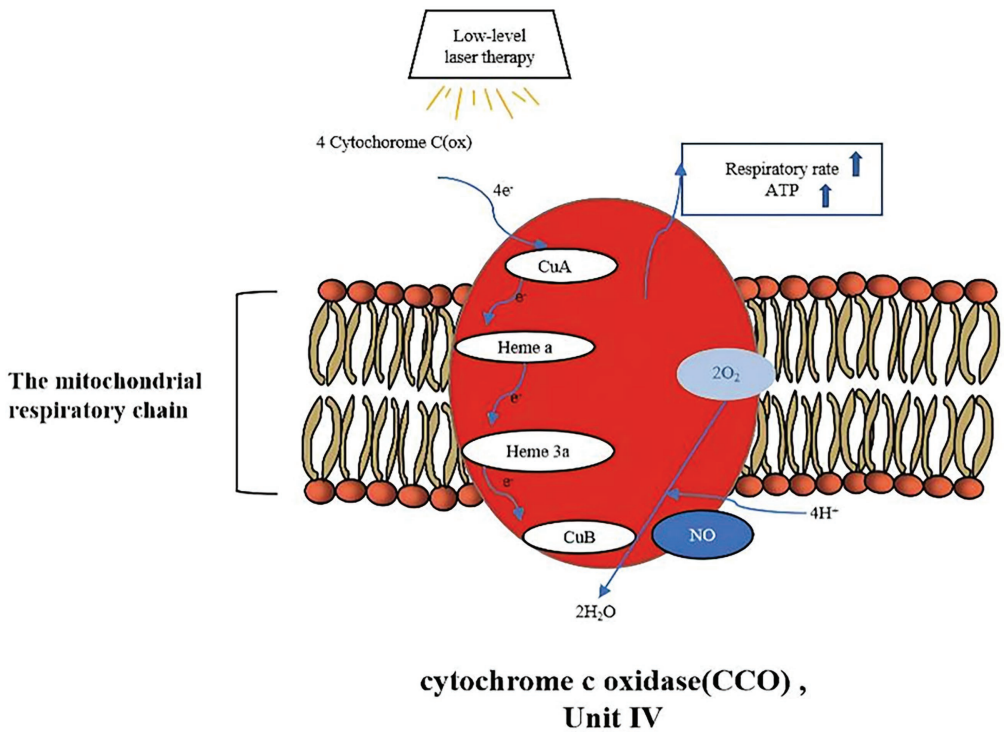

FIGURE 3 | The absorption of photons.

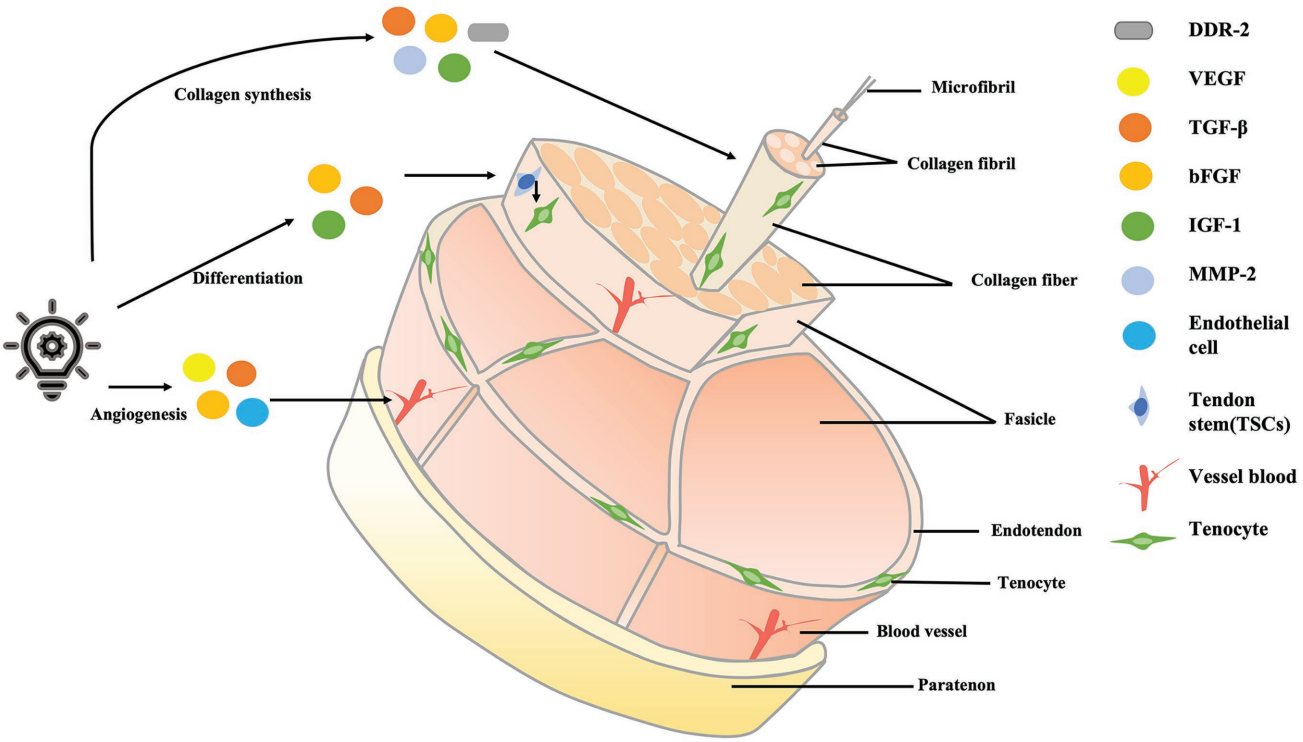

FIGURE 4 | The mechanisms of LLLT in the treatment of tendon injury. LLLT, low-level laser therapy.

TABLE 1 | LLLT regulates the remodeling of ECM.

The remodeling of ECM	Factors associated with the process
Protein synthesis	TGF-β, MMP-2, MMP-3, and MMP-14
Protein degradation	TGF-β, MMP-2, MMP-3, MMP-9, MMP-13, and MMP-14

ECM, extracellular matrix; TGF-β, transforming growth factor-β; MMP-2, matrix metalloprotease 2; MMP-3, matrix metalloprotease 3; MMP-9, matrix metalloprotease 9; MMP-13, matrix metalloprotease 13; and MMP-14, matrix metalloprotease 14.

channels between the neovascularization and circulation systems, accelerating the proliferation and differentiation of endothelial cells, and increasing vascular permeability (Adabbo et al., 2016; Lopes Silva et al., 2020).

Therefore, to meet the requirements of cell oxygenation after the use of LLLT, a series of pro-angiogenic factors (such as VEGF) increase and combine with other cell receptors, the process activates the newly created cells, which then generate new blood vessels. This process achieves the purpose of promoting hemodynamic changes, improving angiogenesis, and tendon injury recovery (Alves et al., 2014; de Freitas and Hamblin, 2016; Wang et al., 2016; Fortuna et al., 2018). While this mechanism has been proposed in much of the literature, experimental evidence is still needed to validate the process. As shown in **Table 2**, LLLT can modulate different cytokines during the injury and repair phases.

LLLT Promotes the Synthesis of Collagen During the Cell Proliferation Phase

The proliferation phase is often accompanied by the formation of a large number of collagen fibers, which are closely related to collagen levels. LLLT can primarily promote collagen synthesis by promoting fibroblast proliferation, which improves the ability of the tendon to heal. Fibroblast activity is mainly related to a multifunctional growth factor-- transforming growth factor-β (TGF-β; Luo et al., 2013). TGF-β is the most effective pro-fibrosis factor in the process of tendon repair and is directly related not only to wound healing but also to scarring during tendon repair; this factor also reduces the number of senescent cells (Sereysky et al., 2013). TGF-β has two main functions. Firstly, it promotes wound healing and scar formation, and secondly, TGF-β plays a key role in muscle fibrosis because it affects changes in ECM-degrading proteases (Luo et al., 2013; Sereysky et al., 2013; Delaney et al., 2017). LLLT can reduce TGF-β content after tendon injury, thus reducing the probability of tendon fibrosis and a series of complications such as tendon tearing after surgery, while also indirectly promoting collagen synthesis (Assis et al., 2013; Delaney et al., 2017). Therefore, LLLT treated muscles have not evidenced excessive scar formation during the repair process and have also shown a large number of regenerating muscle fibers (Luo et al., 2013).

Additionally, fibroblast proliferation is closely related to the collagen receptor—discoidindomainreceptor2 (DDR2), which is regulated by MMP-2. DDR2 collagen receptors regulate fibroblast proliferation and promote ECM synthesis, which is

TABLE 2 | LLLT downregulates diverse inflammatory cytokines.

	Different cytokines associated with an inflammatory response
Damage stage	• M1 macrophages, neutrophils, TNF-α, IL-6, IL-1β, PGE2, Cox-2, and NF-kB pathway
Repair phase	• M2 macrophages, Cox-7, and IL-10

LLLT, low-level laser therapy; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; IL-1β, interleukin-1β; PGE2, prostaglandin E2; Cox-2, cyclooxygenase 2; NF-kB pathway, nuclear factor kappa-B; Cox-7, cyclooxygenase 7; and IL-10, interleukin-10.

important for tendon healing (Illescas-Montes et al., 2019). MMP-2 is an important molecule involved in the regulation of collagen protein synthesis and degradation. LLLT, through the gene expression of MMP-2, enhances its activity and improves the probability of combination with DDR collagen receptors, thereby minimizing damage to the accumulation of collagen, promoting the synthesis of collagen, and improving tendon healing (Abate et al., 2009; Adabbo et al., 2016).

After the proliferation of fibroblasts, collagen synthesis will be affected. A large amount of collagen can be synthesized into collagen fibrils, which are bundled into collagen fibers (Galloway et al., 2013; Wu et al., 2017). After the formation of collagen fibers, the mechanical strength of the tendon can be greatly improved and the tension resistance of injured tissue will also be enhanced, thus indirectly promoting the synthesis of collagen, thus effectively affecting the efficacy of tendon healing (Lock Silveira et al., 2013; Wu et al., 2017).

Studies comparing the diversity of collagen levels after treating tendinopathy with LLLT have shown that the use of LLLT can maximize the contents of type I and III collagen (Lopes Silva et al., 2020). It should be noted, however, that different frequency laser treatments have different effects on tendon repair, among which low-frequency pulsed laser treatment has been shown to maximize the synthesis effect of type I protein, thus regulating the generation of collagen fiber (Guerra et al., 2013).

LLLT Reduces Inflammatory Response During the Tendon Shaping Phase

In general, tendon injury is associated with cytokines such as tumor necrosis factor-α (TNF-α), Interleukin-1β (IL-1β), and Interleukin-10 (IL-10). In the treatment of tendinopathy, LLLT can effectively reduce the content of pro-inflammatory cytokines and also regulate the mRNA expression of anti-inflammatory cytokines (Pires et al., 2011). In the trauma stage, mitochondria are key organelles for activating inflammatory macrophages, which can stimulate M1 macrophages and neutrophils, and produce cytokines such as COX-2, TNF-α, and IL-1β, then activate MAPK and NF- kB pathways, and finally lead to tendon inflammation (Lopes Silva et al., 2020). COX-2 affects the conversion of arachidonic acid into prostaglandins. After 48 h, tendon injury enters the repair stage, during which LLLT can decrease the expression of the NF-kB gene, reduce the activity of COX-2, lower the number of inflammatory mediators and pro-inflammatory factors, and activate M2 macrophages to release anti-inflammatory factors, thus producing anti-inflammatory effects and promoting tendon repair (Rodrigues et al., 2014; Fortuna et al., 2018).

Among these factors, LLLT has a significant effect on TNF-α and IL-6 (Pires et al., 2011; Klatte-Schulz et al., 2018). This is because IL-6 and TNF-α are key cytokines in the development of tendon diseases, and their expressions change with the changes of the tendon (Morita et al., 2017). IL-6 plays a central role in the early stages of tendon injury or after prolonged stress on healthy tendons, and its secretion tends to increase (Legerlotz et al., 2012; Morita et al., 2017). In contrast, LLLT therapy has been shown to significantly reduce the production of pro-inflammatory cytokines other than IL-1β, especially IL-6 mRNA expression (Pires et al., 2011).

Laser treatment at 660 or 870 nm can significantly reduce the expression of TNF-α and IL-6 mRNA, which can significantly reduce the degree of tendon fibrosis and stiffness, maximize the growth capacity of fibroblasts, and improve contraction after tendon injury, thus improving the repairability of muscles (Ryan and Smith, 2007; Mesquita-Ferrari et al., 2011; Pires et al., 2011).

CONCLUSION AND PERSPECTIVES

Tendon injury is a series of muscular imbalances caused by muscle overstrain or poor treatment at the beginning of the disease. Overloading can lead to partial tearing of the tendon initially, and tendon tears are often accompanied by some inflammation and degeneration of the tendon. If not treated in a timely fashion, it will cause structural imbalance and tendon tears, and other consequences (Galloway et al., 2013). It is essentially an unsuccessful healing process, primarily because the inflammatory response destroys the probability of damage repair and is accompanied by several biological changes.

At present, most studies have shown a positive effect of LLLT on tendon repair, especially on some biological factors and structural components in terms of anti-inflammation and analgesia, but this is also dependent on the parameters of the treatment (Bjordal et al., 2006; Lopes Silva et al., 2020). LLLT, also known as photoluminescence, is a non-invasive method that increases the ability to heal part of the damage and to enhance tissue repair without overheating the tissue as the infrared light used in the treatment is not transmitted by an external device, but rather by the body's heat to drive some of the materials to emit infrared to the damaged area (Tsai and Hamblin, 2017; Wickenheisser et al., 2019; Dompe et al., 2020). The process of using LLLT to repair damaged tendons is mainly to exert non-thermal and photochemical reactions in cells to treat the damaged structures. For patients, near-infrared light is commonly used, and LLLT can play its full role under this condition (Wickenheisser et al., 2019). LLLT treatment mainly affects the activity of mitochondria in cells, increasing ATP content, the change of ROS species, and the expression of biological factor mRNA, to stimulate tendon healing (Farivar et al., 2014). Studies have shown that the use of LLLT, combined with certain exercise

therapy such as centrifugal exercise and isometric contraction, can treat tendinopathy to a greater extent than other existing therapies (Jang and Lee, 2012; Girgis and Duarte, 2020).

Based on clinical and animal studies, the mechanism of LLLT promoting tendon injury repair involves reducing the production of inflammatory factors, accelerating the release of anti-inflammatory factors, and promoting angiogenesis. In the phase of promoting angiogenesis, LLLT mainly promotes the production of a series of factors related to angiogenesis through the activation of hypoxia-inducible factors, activates a series of washing, and restores blood function (Xu and Murrell, 2008; Lin et al., 2010; Alves et al., 2014; de Freitas and Hamblin, 2016; Wang et al., 2016; Fortuna et al., 2018; Hamblin, 2018). During the remodeling phase of ECM, LLLT is mainly related to TGF-β and matrix metalloproteinases, and these factors are closely related to the proliferation of fibroblasts, so LLLT regulates the content of different biomolecules, thus promoting collagen synthesis (Mesquita-Ferrari et al., 2011; Assis et al., 2013; Luo et al., 2013; Sereysky et al., 2013; Alves et al., 2014; Delaney et al., 2017; Illescas-Montes et al., 2019). In terms of anti-inflammation, LLLT mainly reduces the expression of the NF-KB gene and COX-2 activity to release a large number of anti-inflammatory factors, so as to achieve the goal of tendon repair (Pires et al., 2011; Deng et al., 2012; Rodrigues et al., 2014; Fortuna et al., 2018). The key factors associated with tendon repair are shown in **Table 3**.

Few studies to date have provided an assessment of the degree of tendon fibrosis after the use of LLLT. TGF-β is the most potent pro-fibrotic factor in the process of tendon healing, and it is directly related to the late scar formation process (Sereysky et al., 2013). While the use of LLLT can inhibit the activity of TGF-β, there have been no definitive studies on LLLT use in this regard. Therefore, it is more likely to cause tendon scarring. Furthermore, the parameters for the use of LLLT present a challenge that continues to be tackled. Presently, there are few clinical criteria for specific conditions, thus further experiments have to be conducted to verify the application of the laser (Lipman et al., 2018).

As incidents of tendon injury are becoming more common, and no definitive, gold standard treatment for tendinopathy has been developed, animal models, despite their differences to humans, will be essential for future research. In the process of using laser treatments for future research, it will be important to strictly compare the differences between control groups and the experimental groups and to accurately control and monitor the frequency levels of low-frequency lasers (Shepherd and Screen, 2013; Hast et al., 2014). In any future study of LLLT, we also need to better ascertain the therapeutic properties and usage specifications of lasers, and further explore the process of LLLT in accelerating cell metabolism, so as to improve the use and efficacy of LLLT in treating tendon injuries.

TABLE 3 | The role of diverse biomolecules.

Different biological factors	Function
TNF-α	• Effective pro-inflammatory cytokines
TGF-β	• Factors involved in wound healing
IL-6	• Key molecules in the early stages of tendon injury and after stress on healthy tendons
MMPs, TIMPs	• The ratio is especially essential, and it has a great influence on the formation of tendon collagen
VEGF	• Vascular endothelial growth factor plays an important role in blood regulation
NF-κB pathway	• Nuclear factor kappa-β, it has a huge impact on the inflammatory response and immune response of cells

TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor-β; IL-6, interleukin-6; MMPs, matrix metalloprotease; TIMPs, tissue inhibitors of metalloproteinases; VEGF, vascular endothelial growth factor; and NF-kB, nuclear factor kappa-β.

AUTHOR CONTRIBUTIONS

KL, XuL, and JL designed the present manuscript. KL drew the manuscript. YC, LJ, BZ, YL, and XiL performed a literature search and selected the studies to be performed. KL, DW, and SL revised the manuscript. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors would like to thank the support of Sub-project of Academician Zhong Shizhen Workstation, Joint cultivation project of Southwest Medical University—The Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University (Osteopathy of Integrated Traditional Chinese and Western Medicine), and Innovation team project of The Affiliated Traditional Chinese Medicine Hospital of Southwest Medical University.

REFERENCES

Abate, M., Silbernagel, K. G., Siljeholm, C., Di Iorio, A., De Amicis, D., Salini, V., et al. (2009). Pathogenesis of tendinopathies: inflammation or degeneration? *Arthritis Res. Ther.* 11:235. doi: 10.1186/ar2723

Adabbo, M., Paolillo, F. R., Bossini, P. S., Rodrigues, N. C., Bagnato, V. S., and Parizotto, N. A. (2016). Effects of low-level laser therapy applied before treadmill training on recovery of injured skeletal muscle in Wistar rats. *Photomed. Laser Surg.* 34, 187–193. doi: 10.1089/pho.2015.4031

Aicale, R., Tarantino, D., and Maffulli, N. (2018). Overuse injuries in sport: a comprehensive overview. *J. Orthop. Surg. Res.* 13:309. doi: 10.1186/s13018-018-1017-5

Alfredson, H., and Cook, J. (2007). A treatment algorithm for managing Achilles tendinopathy: new treatment options. *Br. J. Sports Med.* 41, 211–216. doi: 10.1136/bjsm.2007.035543

Alves, A. N., Santos Fernandes, K. P., Deana, A. M., Bussadori, S. K., and Mesquita-Ferrari, R. A. (2014). Effects of low-level laser therapy on skeletal muscle repair: a systematic review. *Am. J. Phys. Med. Rehabil.* 93, 1073–1085. doi: 10.1097/phm.0000000000000158

Andarawis-Puri, N., Flatow, E. L., and Soslowsky, L. J. (2015). Tendon basic science: development, repair, regeneration, and healing. *J. Orthop. Res.* 33, 780–784. doi: 10.1002/jor.22869

Andres, B. M., and Murrell, G. A. C. (2008). Treatment of tendinopathy: what works, what does not, and what is on the horizon. *Clin. Orthop. Relat. Res.* 466, 1539–1554. doi: 10.1007/s11999-008-0260-1

Assis, L., Soares Moretti, A. I., Abrahao, T. B., de Souza, H. P., Hamblin, M. R., and Parizotto, N. A. (2013). Low-level laser therapy (808 nm) contributes to muscle regeneration and prevents fibrosis in rat tibialis anterior muscle after cryolesion. *Lasers Med. Sci.* 28, 947–955. doi: 10.1007/s10103-012-1183-3

Bjordal, J. M., Lopes-Martins, R. A. B., and Iversen, V. V. (2006). A randomised, placebo controlled trial of low level laser therapy for activated Achilles tendinitis with microdialysis measurement of peritendinous prostaglandin E2 concentrations. *Br. J. Sports Med.* 40, 76–80; discussion 76-80. doi: 10.1136/bjsm.2005.020842

Clijsen, R., Brunner, A., Barbero, M., Clarys, P., and Taeymans, J. (2017). Effects of low-level laser therapy on pain in patients with musculoskeletal disorders: a systematic review and meta-analysis. *Eur. J. Phys. Rehabil. Med.* 53, 603–610. doi: 10.23736/s1973-9087.17.04432-x

de Freitas, L. F., and Hamblin, M. R. (2016). Proposed mechanisms of Photobiomodulation or low-level light therapy. *IEEE J. Sel. Top. Quantum Electron.* 22, 348–364. doi: 10.1109/jstqe.2016.2561201

Delaney, K., Kasprzycka, P., Ciemerych, M. A., and Zimowska, M. (2017). The role of TGF-beta 1 during skeletal muscle regeneration. *Cell Biol. Int.* 41, 706–715. doi: 10.1002/cbin.10725

Deng, B., Wehling-Henricks, M., Villalta, S. A., Wang, Y., and Tidball, J. G. (2012). IL-10 triggers changes in macrophage phenotype that promote muscle growth and regeneration. *J. Immunol.* 189, 3669–3680. doi: 10.4049/jimmunol.1103180

Dompe, C., Moncrieff, L., Matys, J., Grzech-Lesniak, K., Kocherova, I., Bryja, A., et al. (2020). Photobiomodulation-underlying mechanism and clinical applications. *J. Clin. Med.* 9:1724. doi: 10.3390/jcm9061724

Farivar, S., Malekshahabi, T., and Shiari, R. (2014). Biological effects of low level laser therapy. *J. Lasers Med. Sci.* 5, 58–62.

Fortuna, T., Gonzalez, A. C., Sa, M. F., Andrade, Z. D. A., Reis, S. R. A., and Medrado, A. R. A. P. (2018). Effect of 670 nm laser photobiomodulation on vascular density and fibroplasia in late stages of tissue repair. *Int. Wound J.* 15, 274–282. doi: 10.1111/iwj.12861

Galloway, M. T., Lalley, A. L., and Shearn, J. T. (2013). The role of mechanical loading in tendon development, maintenance, injury, and repair. *J. Bone Joint Surg. Am.* 95, 1620–1628. doi: 10.2106/jbjs.L.01004

Genc, E., Yuksel, S., Caglar, A., Beytemur, O., and Gulec, M. A. (2020). Comparison on effects of platelet-rich plasma versus autologous conditioned serum on Achilles tendon healing in a rat model. *Acta Orthop. Traumatol. Turc.* 54, 438–444. doi: 10.5152/j.aott.2020.18498

Girgis, B., and Duarte, J. A. (2020). Physical therapy for tendinopathy: an umbrella review of systematic reviews and meta-analyses. *Phys. Ther. Sport* 46, 30–46. doi: 10.1016/j.ptsp.2020.08.002

Guerra, F. D. R., Vieira, C. P., Almeida, M. S., Oliveira, L. P., de Aro, A. A., and Pimentel, E. R. (2013). LLLT improves tendon healing through increase of MMP activity and collagen synthesis. *Lasers Med. Sci.* 28, 1281–1288. doi: 10.1007/s10103-012-1236-7

Hamblin, M. R. (2018). Mechanisms and mitochondrial redox signaling in photobiomodulation. *Photochem. Photobiol.* 94, 199–212. doi: 10.1111/php.12864

Hast, M. W., Zuskov, A., and Soslowsky, L. J. (2014). The role of animal models in tendon research. *Bone Joint Res.* 3, 193–202. doi: 10.1302/2046-3758.36.2000281

Illescas-Montes, R., Melguizo-Rodriguez, L., Garcia-Martinez, O., de Luna-Bertos, E., Javier Manzano-Moreno, F., Ruiz, C., et al. (2019). Human fibroblast gene expression modulation using 940 NM diode laser. *Sci. Rep.* 9:12037. doi: 10.1038/s41598-019-48595-2

Jang, H., and Lee, H. (2012). Meta-analysis of pain relief effects by laser irradiation on joint areas. *Photomed. Laser Surg.* 30, 405–417. doi: 10.1089/pho.2012.3240

Kaux, J.-F., Forthomme, B., Le Goff, C., Crielaard, J.-M., and Croisier, J.-L. (2011). Current opinions on tendinopathy. *J. Sports Sci. Med.* 10, 238–253.

Klatte-Schulz, F., Minkwitz, S., Schmock, A., Bormann, N., Kurtoglu, A., Tsitsilonis, S., et al. (2018). Different Achilles tendon pathologies show distinct histological and molecular characteristics. *Int. J. Mol. Sci.* 19:404. doi: 10.3390/ijms19020404

Legerlotz, K., Jones, E. R., Screen, H. R. C., and Riley, G. P. (2012). Increased expression of IL-6 family members in tendon pathology. *Rheumatology* 51, 1161–1165. doi: 10.1093/rheumatology/kes002

Li, H.-Y., and Hua, Y.-H. (2016). Achilles tendinopathy: current concepts about the basic science and clinical treatments. *Biomed. Res. Int.* 2016:6492597. doi: 10.1155/2016/6492597

Lin, F., Josephs, S. F., Alexandrescu, D. T., Ramos, F., Bogin, V., Gammill, V., et al. (2010). Lasers, stem cells, and COPD. *J. Transl. Med.* 8:16. doi: 10.1186/1479-5876-8-16

Lipman, K., Wang, C., Ting, K., Soo, C., and Zheng, Z. (2018). Tendinopathy: injury, repair, and current exploration. *Drug Des. Devel. Ther.* 12, 591–603. doi: 10.2147/dddt.S154660

Lock Silveira, P. C., da Silva, L. A., Pinho, C. A., De Souza, P. S., Ronsani, M. M., Scheffer, D. D. L., et al. (2013). Effects of low-level laser therapy (GaAs) in an animal model of muscular damage induced by trauma. *Lasers Med. Sci.* 28, 431–436. doi: 10.1007/s10103-012-1075-6

Lopes Silva, R. S. D., Pessoa, D. R., Mariano, R. R., Castro, A. B. S., de Oliveira, R. A., and Ferraresi, C. (2020). Systematic review of photobiomodulation therapy (PBMT) on the experimental calcaneal tendon injury in rats. *Photochem. Photobiol.* 96, 981–997. doi: 10.1111/php.13262

Luo, L., Sun, Z., Zhang, L., Li, X., Dong, Y., and Liu, T. C.-Y. (2013). Effects of low-level laser therapy on ROS homeostasis and expression of IGF-1 and TGF-beta 1 in skeletal muscle during the repair process. *Lasers Med. Sci.* 28, 725–734. doi: 10.1007/s10103-012-1133-0

Mesquita-Ferrari, R. A., Martins, M. D., Silva, J. A. Jr., da Silva, T. D., Piovesan, R. F., Santos Pavesi, V. C., et al. (2011). Effects of low-level laser therapy on expression of TNF-alpha and TGF-beta in skeletal muscle during the repair process. *Lasers Med. Sci.* 26, 335–340. doi: 10.1007/s10103-010-0850-5

Morita, W., Dakin, S. G., Snelling, S. J. B., and Carr, A. J. (2017). Cytokines in tendon disease A SYSTEMATIC REVIEW. *Bone Joint Res.* 6, 656–664. doi: 10.1302/2046-3758.612.Bjr-2017-0112.R1

Nogueira Junior, A. C., and Moura Junior, M. D. J. (2015). The effects of laser treatment in tendinopathy: a systematic review. *Acta Ortop. Bras.* 23, 47–49. doi: 10.1590/1413-78522015230100513

Piccionello, A. P., Riccio, V., Senesi, L., Volta, A., Pennasilico, L., Botto, R., et al. (2021). Adipose micro-grafts enhance tendinopathy healing in ovine model: an in vivo experimental perspective study. *Stem Cells Transl. Med.* 10, 1544–1560. doi: 10.1002/sctm.20-0496

Pires, D., Xavier, M., Araujo, T., Silva, J. A. Jr., Aimbire, F., and Albertini, R. (2011). Low-level laser therapy (LLLT; 780 nm) acts differently on mRNA expression of anti- and pro-inflammatory mediators in an experimental model of collagenase-induced tendinitis in rat. *Lasers Med. Sci.* 26, 85–94. doi: 10.1007/s10103-010-0811-z

Ragni, E., Papait, A., Perucca Orfei, C., Silini, A. R., Colombini, A., Vigano, M., et al. (2021). Amniotic membrane-mesenchymal stromal cells secreted factors and extracellular vesicle-miRNAs: anti-inflammatory and regenerative features for musculoskeletal tissues. *Stem Cells Transl. Med.* 10, 1044–1062. doi: 10.1002/sctm.20-0390

Rand, S. E., Goerlich, C., Marchand, K., and Jablecki, N. (2007). The physical therapy prescription. *Am. Fam. Physician* 76, 1661–1666.

Rodrigues, N. C., Brunelli, R., Abreu, D. C. C., Fernandes, K., Parizotto, N. A., and Renno, A. C. M. (2014). Morphological aspects and Cox-2 expression after exposure to 780-nm laser therapy in injured skeletal muscle: an in vivo study. *Braz. J. Phys. Ther.* 18, 395–401. doi: 10.1590/bjpt-rbf.2014.0057

Ryan, T., and Smith, R. K. W. (2007). An investigation into the depth of penetration of low level laser therapy through the equine tendon in vivo. *Ir. Vet. J.* 60, 295–299. doi: 10.1186/2046-0481-60-5-295

Sandona, M., Di Pietro, L., Esposito, F., Ventura, A., Silini, A. R., Parolini, O., et al. (2021). Mesenchymal stromal cells and their secretome: new therapeutic perspectives for skeletal muscle regeneration. *Front. Bioeng. Biotechnol.* 9:652970. doi: 10.3389/fbioe.2021.652970

Sereysky, J. B., Flatow, E. L., and Andarawis-Puri, N. (2013). Musculoskeletal regeneration and its implications for the treatment of tendinopathy. *Int. J. Exp. Pathol.* 94, 293–303. doi: 10.1111/iep.12031

Shen, H., Yoneda, S., Abu-Amer, Y., Guilak, F., and Gelberman, R. H. (2020). Stem cell-derived extracellular vesicles attenuate the early inflammatory response after tendon injury and repair. *J. Orthop. Res.* 38, 117–127. doi: 10.1002/jor.24406

Shepherd, J. H., and Screen, H. R. C. (2013). Fatigue loading of tendon. *Int. J. Exp. Pathol.* 94, 260–270. doi: 10.1111/iep.12037

Stania, M., Juras, G., Chmielewska, D., Polak, A., Kucio, C., and Krol, P. (2019). Extracorporeal shock wave therapy for Achilles tendinopathy. *Biomed. Res. Int.* 2019, 1–13. doi: 10.1155/2019/3086910

Thomopoulos, S., Parks, W. C., Rifkin, D. B., and Derwin, K. A. (2015). Mechanisms of tendon injury and repair. *J. Orthop. Res.* 33, 832–839. doi: 10.1002/jor.22806

Tsai, S.-R., and Hamblin, M. R. (2017). Biological effects and medical applications of infrared radiation. *J. Photochem. Photobiol. B* 170, 197–207. doi: 10.1016/j.jphotobiol.2017.04.014

Wang, X., Tian, F., Soni, S. S., Gonzalez-Lima, F., and Liu, H. (2016). Interplay between up-regulation of cytochrome-c-oxidase and hemoglobin oxygenation induced by near-infrared laser. *Sci. Rep.* 6:30540. doi: 10.1038/srep30540

Wickenheisser, V. A., Zywot, E. M., Rabjohns, E. M., Lee, H. H., Lawrence, D. S., and KathleenTarrant, T. (2019). Laser light therapy in inflammatory, musculoskeletal, and autoimmune disease. *Curr Allergy Asthma Rep* 19:37. doi: 10.1007/s11882-019-0869-z

Wu, F., Nerlich, M., and Docheva, D. (2017). Tendon injuries: basic science and new repair proposals. *EFORT Open Rev.* 2, 332–342. doi: 10.1302/2058-5241.2.160075

Xu, Y., and Murrell, G. A. C. (2008). The basic science of tendinopathy. *Clin. Orthop. Relat. Res.* 466, 1528–1538. doi: 10.1007/s11999-008-0286-4

Zein, R., Selting, W., and Hamblin, M. R. (2018). Review of light parameters and photobiomodulation efficacy: dive into complexity. *J. Biomed. Opt.* 23, 1–17. doi: 10.1117/1.Jbo.23.12.120901

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frontiers in Physiology

SYSTEMATIC REVIEW
published: 17 February 2022
doi: 10.3389/fphys.2022.838069

Changes in Cytokines Concentration Following Long-Distance Running: A Systematic Review and Meta-Analysis

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OPEN ACCESS

Edited by:
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Reviewed by:
Luis Manuel Rama, University of Coimbra, Portugal
Jesús Rodríguez Huertas, University of Granada, Spain

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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *17 December 2021*
Accepted: *25 January 2022*
Published: *17 February 2022*

Citation:
Alves MDdJ, Silva DdS, Pereira EVM, Pereira DD, de Sousa Fernandes MS, Santos DFC, Oliveira DPM, Vieira-Souza LM, Aidar FJ and de Souza RF (2022) Changes in Cytokines Concentration Following Long-Distance Running: A Systematic Review and Meta-Analysis. Front. Physiol. 13:838069. doi: 10.3389/fphys.2022.838069

Long-distance running is an exhausting effort for the whole organism. Prolonged aerobic exercise induces changes in inflammatory markers. However, predicting muscle damage in response has limitations in terms of selecting biomarkers used to measure inflammatory status. The present study conducts a systematic review and meta-analysis of articles focusing in ultra-marathon, marathon, and half-marathon and levels of cytokines. The search was conducted in PubMed, Web of Science, and Scopus databases, resulting in the inclusion of 76 articles. IL-6 was highlighted, evaluated in 62 studies and show increase in the standard mean difference (SMD): half-marathon (SMD −1.36; IC 95%: −1.82, −0.89, Ch^2:0.58; tau^2:0.00; $p < 0.0001$), marathon (SMD −6.81; IC 95%: −9.26, −4.37; Ch^2:481.37 tau^2:11.88; $p < 0.0001$) and ultra-marathon (SMD −8.00 IC 95%: −10.47, −5.53; Ch^2:328.40; tau^2:14.19; $p < 0.0001$). In contrast meta-regression analysis did not show relationship to the running distance ($p = 0.864$). The meta-analysis evidenced increase in the concentration of IL-1ra ($p < 0.0001$), IL-1B ($p < 0.0001$), IL-8 ($p < 0.0001$), IL-10 ($p < 0.0001$) and TNF-α ($p < 0.0001$). Reduction in IL-2 ($p < 0.0001$) and INF-y ($p < 0.03$) and no change in the IL-4 ($p < 0.56$). The number of studies evaluating the effect of adipokines was limited, however Leptin and Resistin were recurrent. The effects of an acute bout of prolonged aerobic exercise will protect against chronic systemic inflammation. The time to return to baseline values showed a substantial and dose-dependent relationship with run volume. The concentration of IL-6 was robustly studied and the marathon running was the most explored. Network of endocrine interactions in which circulating factors, released in extreme exercises,

interplay through inter-organ crosstalk and physiologic changes were expressed. The running volume variability was able to modulate compounds that play a fundamental role in the maintenance of homeostasis and cell signaling.

Keywords: marathon, aerobic, endurance, cytokine, myokine, adipokine

INTRODUCTION

Regular physical activity has been described as positive on protein and enzyme concentration, increases in insulin sensitivity, skeletal muscle glucose uptake (Turcotte and Fisher, 2008) and also for promoting a generalized anti-inflammatory state (Gleeson et al., 2011). In contrast, some negative effects are evidenced after complex competitions, resulting from the high volume and repeated physical efforts as occurs in ultra-marathons (>6 h of duration or >50 km) (Knechtle and Nikolaidis, 2018). These specific runs require great resistance and muscle contraction, resulting in microtraumas to the connective tissue, bone, and skeletal muscle with exercise-induced muscle damage (EIMD) (Smith, 2000; Suzuki et al., 2003; Järvinen et al., 2013).

The EIMD is related to the inflammatory response, characterized by the body's defense against an aggressor agent, whose objective is to promote repair of the damaged tissue (Cerqueira et al., 2020). The magnitude of this process is regulated by pro (about 1.5−24 h after exercise) and anti-inflammatory factors (from 24 to 72 h after exercise) (Zaldivar et al., 2006; Allen et al., 2015; Cerqueira et al., 2020), with cytokines being responsible for coordination, amplification, regulation of the magnitude, duration, and effect of inflammatory events (Moldoveanu et al., 2001). Cytokines are molecules produced by cells of the immune system, active musculature, and other tissues such as adipose tissue (de Oliveira dos Santos et al., 2021). In addition to an important modulating activity of inflammation, they regulate the activation of energy pathways that support this process (Petersen and Pedersen, 2005).

Adipose and skeletal muscle and tissue are the main endocrine organs that produce adipokines and myokines. These biomarkers can be detrimental or beneficial in the body and crosstalk between different tissues (Leal et al., 2018) acting on the endocrine, paracrine and autocrine pathways (de Oliveira dos Santos et al., 2021). Knowledge about the loss of cytokine homeostasis brings to light a better understanding of the metabolic disorders resulting from long-term running, resulting to marked changes in the concentration profile, which can be the basis of many physiological and pathophysiological disorders (Knechtle and Nikolaidis, 2018), questioning the real health benefit. These questions are relevant not only in ultra-marathons, but also in less volumes (i.e., marathons and half-marathons) (Suzuki et al., 2003; Kaufmann et al., 2021; Tanner et al., 2021).

Under normal conditions cytokines lead to a systemic anti-inflammatory state (Gomarasca et al., 2020). Becoming potentially permissive for optimizing body energy expenditure (Pedersen, 2019; Das et al., 2020) and for the protection of diseases associated with inflammation, insulin resistance and hyperlipidemia. On the other hand, alarming results are shown when exercise is performed in large volumes, a dramatic increase in interleukin (IL)-6 concentration is observed (Margeli et al., 2005), a decrease in the concentration of myostatin mRNA in the skeletal muscle (Allen et al., 2011), consistent acute tissue inflammatory lesion (Margeli et al., 2005; Papassotiriou et al., 2008; Goussetis et al., 2009) and EIMD (Suzuki et al., 2003). That is, indicators of the potentially more injurious condition in prolonged exercise (Kerschan-Schindl et al., 2009).

Understanding that broadening the discussion of this information is of great importance for sport physiologists, coaches and athletes, which show an increase in the number of practitioners and popularity. In this systematic review followed by a meta-analysis, we sought to verify the current state of investigation in relation to the effects of long-term running on cytokine concentration.

METHODS

Search Strategy

The systematic review report was carried out based on the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement" (PRISMA) (Page et al., 2021). A research was performed in the PubMed, Web of Science and Scopus databases, from September 1 to 13, 2021, using the following boolean operators (AND/OR) and terms: "marathon" OR "aerobic" OR "endurance" AND "cytokine" OR "myokine" OR "adipokine", as well as the use of synonyms and related search terms, with no start date limit and, considering as the final year, the year 2021. The strategy of seeking additional articles in the gray literature and in the references of the papers found were adopted, with the aim of expanding the results.

Search results loaded into the online bibliographic management software Rayyan™. After excluding duplicates, all titles and abstracts were independently analyzed by two investigators to determine the study's eligibility for inclusion in the review, in case of divergence a third author was consulted to establish a consensus. After these initial steps, the full texts were evaluated and the name of the first author, year of publication, title, objective, running distance, sample, runner's level, age, type of collection and inflammatory markers (pre, immediately after, after 24, 48, and 72 h).

Eligibility Criteria

Abstract Selection

During the process of reading titles and abstracts, the following inclusion criteria were adopted: (I) studies involving running with distances equal to or >21 km and (II) measurement of pre and post-running inflammatory biomarkers.

Full-Text Articles Selection

As a second selection step, the studies were excluded for the following reasons: (1) pre-running collections with periods >7 days, (2) post-running with only periods >24 h, (3) jobs that showed running on a treadmill, (4) ingestion of drug, supplement or performance-maximizing drink, (5) runners with some pathology and (6) another sporting activity added to running. All parameters were evaluated in blood, urine, nasal and sputum samples collected before and after running.

Risk of Bias Assessment

The recommendations of the Cochrane risk of bias assessment tool were followed, adopting the risk of bias strategy (**Table 1**) by two authors independently, and a third author was consulted to define the differences (Higgins et al., 2011) and the Review Manager program (RevMan5.3), developed for Systematic Reviews, which is available for free download (https://training.cochrane.org/online-learning/core-software-cochrane-reviews/revman/revman-5-download).

Statistical Analysis

The results were expressed as the standard mean difference (SMD) of the 95% confidence intervals (CI) presented by the Forest plot graph. For the analysis, 2 conditions were used: pre and post running. The studies that evaluated IL-6 were combined for trials with parameters (half-marathon, marathon, and ultra-marathon). To identify if the variables ultra-marathon, marathon and half-marathon could be significantly associated with effect size differences, a meta-regression analysis was performed. For studies with more than one intervention group, we considered only the control or placebo group without drug administration or other interventions. We used Cochran's (Ch^2) and tau-square test (tau^2) to assess heterogeneity. The I^2 statistic was used to assess inconsistency (the percentage of the total variation of heterogeneity) of the effects of exercise (Higgins et al., 2003). We assessed visually and objectively the propagation of the risk of bias the symmetry graphically using funnel plots. The threshold for statistical significance was set to $p < 0.05$.

RESULTS

Search Results

In the initial search, 5,528 articles were found, and 2,372 duplicate articles were excluded. Of 3,156 articles screened for eligibility, 3,061 were excluded based on title or abstract. The full texts of 95 potentially eligible studies were evaluated. Of these, 76 met the criteria and were included in the review, among which 29 made up the meta-analyses (**Figure 1**).

Risk of Bias of Included Studies

Of the 29 studies included, at least 10 studies were at risk of bias. Four studies had a high risk of random sequence generation and allocation concealment. Ten studies showed risk in blinding participants and researchers. When the blinding of results and selective notification was evaluated, two studies presented a risk. All studies had a low risk for incomplete outcome data. No study presented the risk of other biases (**Figures 2**, **3**).

Running Characteristic

Table 2 summarizes the results obtained from the 76 studies that investigated the relation to the degree of running volume in studies that evaluated cytokines in ultra-marathon, marathon, and half-marathon competitions. It was identified that the marathon had the highest proportion of articles investigated 39/76, followed by running of ultra-marathon 21/76 and half-marathon 9/76, four studies evaluated marathon and half-marathon (Bonsignore et al., 2002; Reihmane et al., 2013; Niemelä et al., 2016; Bekos et al., 2019), furthermore 2/76 evaluated the distance of 35 km (Miles et al., 2006; Yargic et al., 2019) and the study of Skinner et al. (2021) evaluated two runs 40 e 171 km.

From the articles that evaluated ultra-marathon running, four studies (Drenth et al., 1995; Donnikov et al., 2009; Gill et al., 2015a; Benedetti et al., 2021) evaluated distances in the ranges of 51–86, 51–81, 122–208, and 99–218 km respectively, two studies (Mastaloudis et al., 2004; Díaz-Castro et al., 2012) 50 km distance, one study (Sansoni et al., 2017) 65 km distance, one study (Nieman et al., 2002) 80 km distance, one study (Peters et al., 2001) 90 km distance, four studies (Chiu et al., 2013; Czajkowska et al., 2020; Kasprowicz et al., 2020; Wołyniec et al., 2020) evaluated 100 km, four studies (Nieman et al., 2003, 2005, 2006, 2007) evaluated 160 km, one study (Roupas et al., 2013) evaluated 180 km, one study (Kim et al., 2007) evaluated 200 km, one study (Gill et al., 2015b) evaluated 230 km and one study (Shin and Lee, 2013) evaluated 308 km. Some studies that evaluated the ultra-marathons presented particularities in relation to the topography characteristic. Four studies were carried out in mountains with uphill (5.500 meters) and dowhill (6.500 meters) (Nieman et al., 2003, 2005, 2006 and Nieman et al., 2007). Three studies reported parts with uphill by 2,800 meters (Díaz-Castro et al., 2012); 4,000 meters (Sansoni et al., 2017) and 10000 meters (Skinner et al., 2021). Four studies on trails with the varying ground (Mastaloudis et al., 2004; Roupas et al., 2013; Gill et al., 2015a,b). Five studies with flat ground, including athletics tracks (Chiu et al., 2013; Czajkowska et al., 2020; Kasprowicz et al., 2020; Wołyniec et al., 2020; Benedetti et al., 2021), the other studies did not identify the running ground.

Runner's Level and Sex

Experienced runners, athletes, trained and well-trained were evaluated in 22 studies (Niess et al., 1999; Fehrenbach et al., 2000; Ostrowski et al., 2000; Suzuki et al., 2000, 2003; Toft et al., 2000; Bonsignore et al., 2002; Zaccaria et al., 2002; Nieman et al., 2003, 2005, 2006, 2007; Cox et al., 2010; Abbasi et al., 2013; Chiu et al., 2013; Roupas et al., 2013; Santos et al., 2013b; Shanely et al., 2014; Bachi et al., 2015; Sansoni et al., 2017; Passos et al., 2019; Gaggini et al., 2021). Amateur, recreational runners were present in 13 studies (Drenth et al., 1995; Castell et al., 1996; Starkie et al., 2001; Bonsignore et al., 2002; Kim et al., 2007; Díaz-Castro et al., 2012; Vaisberg et al., 2012, 2013; Reihmane et al., 2013; Vuolteenaho et al., 2014; Luna Junior et al., 2016; dos Santos et al., 2020; Sliwicka et al., 2021). Other characteristics such as experience ranging from 1 to 16 years, finalists, and time in a marathon running of <5 h were adopted in three studies

TABLE 1 | Risk of bias evaluation of included studies.

Bias domain	Source of bias	Support judgment
Selection bias	Random sequence generation	The method used to generate the allocation sequence had sufficient detail to allow an evaluation and produce comparable groups
	Allocation concealment	The method used to conceal the allocation sequence, or detailing the intervention allocations could have been predicted
Bias performance	Blindness of participants	There was blind trial for participants and researchers
Bias detection	Result evaluation blindness	The measures used for the evaluation of results were blind
Frequency of friction	Incomplete results data	The conclusion of the results presented exclusions of analyzes or any other friction
Report Bias	Selective reports	How the selective results report was examined and what was found
Another type of bias	Anything else, ideally pre-specified	Important concerns about bias not covered in the other domains in the tool

respectively (Uchakin et al., 2003; Pugh et al., 2019; Wołyniec et al., 2020). The other studies did not presented the levels of the evaluated runners. Women were present in 35% of the studies. However, only the study by Abbasi et al. (2013) broken down the results by sex.

Running Time

Mean running conclusion times were reported in 68% of the studies. In the half-marathon, the mean times varied between 1:30 and 2:12 h (Zaccaria et al., 2002; Ng et al., 2008; Cox et al., 2010; Abbasi et al., 2013; Reihmane et al., 2013; Niemelä et al., 2016; Costello et al., 2020; Gaggini et al., 2021). In marathons the mean times varied between 2:52 and 4:41 h (Castell et al., 1996; Toft et al., 2000; Pistilli et al., 2002; Henson et al., 2004; Howatson et al., 2010; Scherr et al., 2011, 2012; Nickel et al., 2012; Bernecker et al., 2013; Reihmane et al., 2013; Santos et al., 2013a,b; Vaisberg et al., 2013; Shanely et al., 2014; Vuolteenaho et al., 2014; Wilhelm et al., 2014; Niemelä et al., 2016; Clifford et al., 2017; Passos et al., 2019; Pugh et al., 2019; Sierra et al., 2019a; Batatinha et al., 2020; dos Santos et al., 2020; Larsen et al., 2020; Sliwicka et al., 2021; Tavares-Silva et al., 2021). In ultra-marathons, the mean times ranged between 6:00 and 62:20 h (Drenth et al., 1995; Peters et al., 2001; Nieman et al., 2002, 2003, 2005, 2006, 2007; Zaccaria et al., 2002; Mastaloudis et al., 2004; Kim et al., 2007; Donnikov et al., 2009; Chiu et al., 2013; Shin and Lee, 2013; Gill et al., 2015a; Czajkowska et al., 2020; Wołyniec et al., 2020; Benedetti et al., 2021; Skinner et al., 2021), with distances of 51–308 km. Three studies evaluated the specific distances of 35, 35.2 and 40 km, with the following mean conclusion times 5:08 (Yargic et al., 2019), 6:10 (Miles et al., 2006) and 6:50 hours (Skinner et al., 2021) respectively.

Effects of Long-Distance Running on Cytokine Concentration

The effects of long-distance running on cytokine concentration were evaluated in 76 studies. The analyzed results comprised a period of up to 72 h after the running. The concentration of 35 cytokines was identified (IL-6, IL-8, IL-1β, IL-1ra, IL-2, IL-4, IL-10, IL-12, IL-12p40, IL-12p70, IL-23, IL-33, IL-15, IL-7, IL-17a, IL-3, IL-5, Tumor Necrosis Factor-Alfa (TNF-α), Interferon Gamma (IFN-γ), Granulocyte Colony-Stimulating Factor (VEGF-A), Fractalkine, Leptin, Resistin, Adiponectin, Visfatin, Tumor Necrosis Factor Type II p75 (sTNFRII), Interleukin-6 Receptor gp80 (sIL-6R), Transforming Growth Factor-Beta (TGF-β), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Granulocyte Colony-Stimulating Factor (G-CSF), Monocyte Chemotactic Protein 1 (MCP-1), Macrophage Inflammatory Protein 1 Beta (MIP-1β), Heat Shock Protein (HSP)-72, HSP27 and HPS70). The review shows that long-term runs can change the volume of cytokine concentration, presenting as relevant factors the distance of the run and the recovery time until the analysis (**Table 2**).

Interleukin 6

The most evaluated cytokine was IL-6 found in 62 studies (Drenth et al., 1995; Castell et al., 1996; Ostrowski et al., 1998, 1999, 2000; Neidhart et al., 2000; Suzuki et al., 2000, 2003; Toft et al., 2000; Nieman et al., 2001, 2002, 2003, 2005, 2006, 2007; Starkie et al., 2001; Bonsignore et al., 2002; Pistilli et al., 2002; Uchakin et al., 2003; Mastaloudis et al., 2004; Miles et al., 2006; Kim et al., 2007; Siegel et al., 2007; Ng et al., 2008; Donnikov et al., 2009; Cox et al., 2010; Howatson et al., 2010; Scherr et al., 2011, 2012; Díaz-Castro et al., 2012; Nickel et al., 2012; Vaisberg et al., 2012, 2013; Abbasi et al., 2013; Bernecker et al., 2013; Chiu et al., 2013; Reihmane et al., 2013; Santos et al., 2013a, 2016; Shin and Lee, 2013; Wilhelm et al., 2014; Gill et al., 2015a,b; Luna Junior et al., 2016; Niemelä et al., 2016; Zimmer et al., 2016; Clifford et al., 2017; Passos et al., 2019; Pugh et al., 2019; Sierra et al., 2019a,b; Yargic et al., 2019; Batatinha et al., 2020; Costello et al., 2020; dos Santos et al., 2020; Kasprowicz et al., 2020; Larsen et al., 2020; Wołyniec et al., 2020; Benedetti et al., 2021; Gaggini et al., 2021; Skinner et al., 2021; Sliwicka et al., 2021). Of this total, only the studies of Reihmane et al. (2013) and Costello et al. (2020) (3%) did not show increased concentration of IL-6 after half-marathon running.

Interleukin 1β

Nineteen studies were found that evaluated the IL-1β (Drenth et al., 1995; Ostrowski et al., 1998, 1999; Neidhart et al., 2000; Suzuki et al., 2000, 2003; Nieman et al., 2001; Uchakin et al., 2003; Ng et al., 2008; Gill et al., 2015a,b; Luna Junior et al., 2016; Santos et al., 2016; Clifford et al., 2017; Passos et al., 2019; Sierra et al., 2019a; Batatinha et al., 2020; dos Santos et al., 2020; Skinner et al., 2021). In these studies, 20 runs were evaluated [half-marathon ($n = 1$), ultra-marathon ($n = 4$), marathon (n

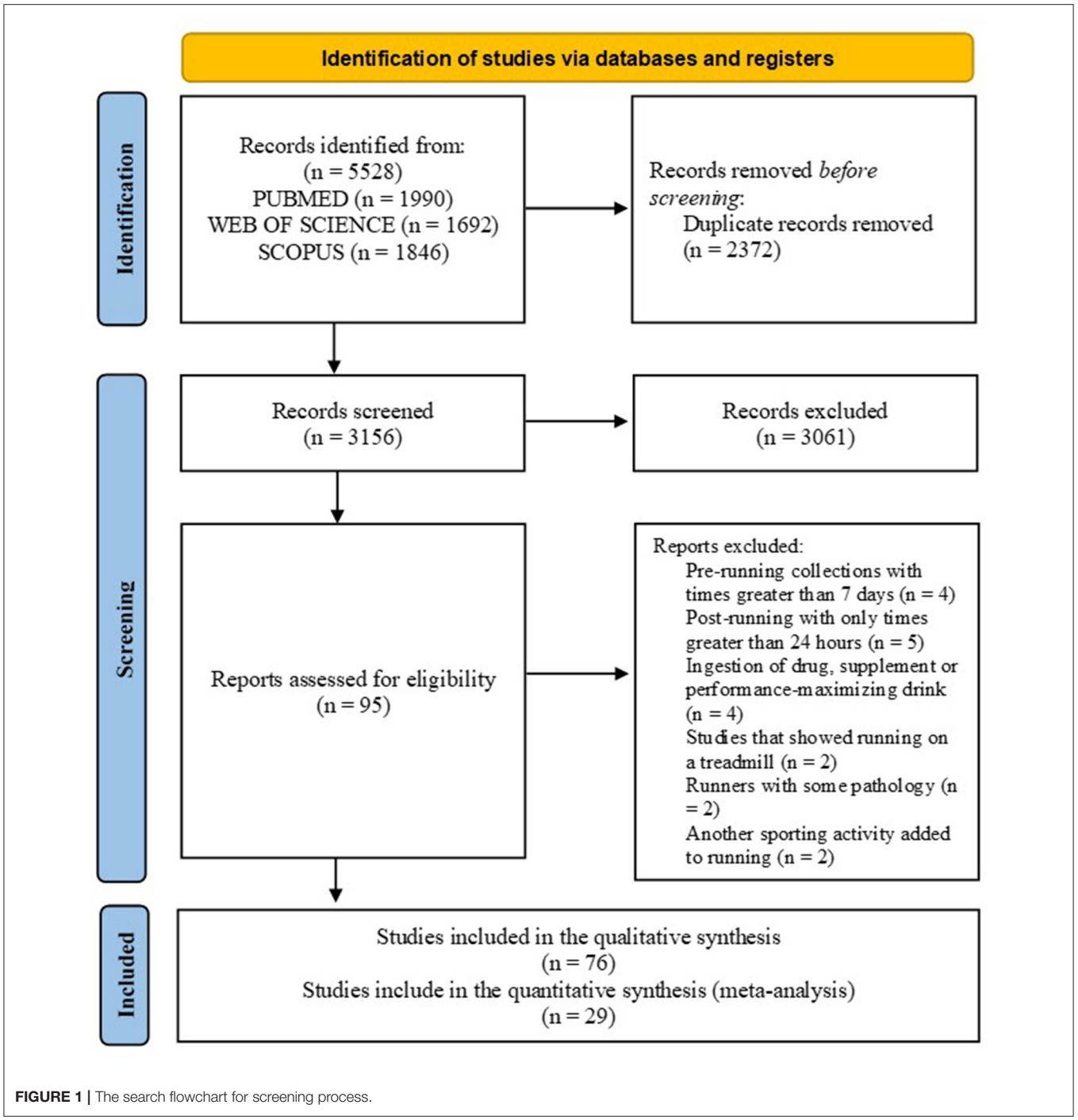


FIGURE 1 | The search flowchart for screening process.

= 14) and 40 km running (n = 1)], of this total, (100%) half-marathon (Ng et al., 2008) and 40 km (Skinner et al., 2021), (50%) marathon (Neidhart et al., 2000; Suzuki et al., 2000; Uchakin et al., 2003; Luna Junior et al., 2016; Santos et al., 2016; Batatinha et al., 2020; dos Santos et al., 2020) e (25%) ultra-marathon (Drenth et al., 1995), did not show increased concentration of IL-1β. When the behavior of IL-1β between two tests was analyzed (40 km vs 171 km) in the same study, IL-1β was increased only in the ultra-marathon running (Skinner et al., 2021).

Interleukin-1 Receptor Antagonist

Twenty-three studies were found that evaluated interleukin-1 receptor antagonist (IL-1ra), in all run volumes evaluated (Drenth et al., 1995; Castell et al., 1996; Ostrowski et al., 1998, 1999, 2000; Neidhart et al., 2000; Suzuki et al., 2000; Toft et al., 2000; Nieman et al., 2001, 2002, 2003, 2005, 2006, 2007; Peters et al., 2001; Pistilli et al., 2002; Ng et al., 2008; Cox et al., 2010; Díaz-Castro et al., 2012; Abbasi et al., 2013; Santos et al., 2013a; Gill et al., 2015b; Clifford et al., 2017). Of this total, only the

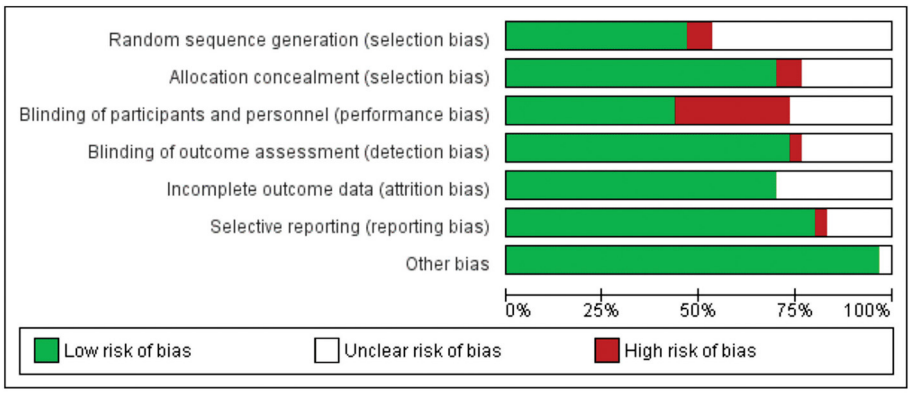


FIGURE 2 | Risk of bias evaluation of included studies.

study of Clifford et al. (2017) (3%) showed no elevation in the concentration of IL-1ra after marathon running.

Interleukin 2

Eight studies were found that evaluated IL-2, in two runs studied, being six marathons (Castell et al., 1996; Suzuki et al., 2000; Santos et al., 2013b; Clifford et al., 2017; Batatinha et al., 2020; Tavares-Silva et al., 2021) and two ultra-marathon (Nieman et al., 2002; Skinner et al., 2021). Of this total, the studies of Castell et al. (1996), Suzuki et al. (2000) and Clifford et al. (2017) showed no decrease in IL-2 concentration and the studies by Nieman et al. (2002) and Skinner et al. (2021) demonstrated an increase in IL-2 concentration.

Interleukin 4

Seven studies were found that evaluated IL-4 (Suzuki et al., 2000; Luna Junior et al., 2016; Clifford et al., 2017; Batatinha et al., 2020; dos Santos et al., 2020; Skinner et al., 2021; Tavares-Silva et al., 2021). Of this total, only the study of Skinner et al. (2021) found an increase in IL-4 after a 171 km run.

Interleukin 8

Twenty-seven studies were found that evaluated IL-8, in the distances between half-marathon and ultra-marathon running ($\leq$ 208 km) (Niess et al., 1999; Fehrenbach et al., 2000; Suzuki et al., 2000, 2003; Nieman et al., 2001, 2002, 2003, 2005, 2006, 2007; Pistilli et al., 2002; Cox et al., 2010; Abbasi et al., 2013; Shanely et al., 2014; Bachi et al., 2015; Gill et al., 2015a; Luna Junior et al., 2016; Niemelä et al., 2016; Santos et al., 2016; Clifford et al., 2017; Passos et al., 2019; Pugh et al., 2019; Sierra et al., 2019a,b; Batatinha et al., 2020; dos Santos et al., 2020; Skinner et al., 2021). Of this total, only the studies of Bachi et al. (2015), Luna Junior et al. (2016) and Sierra et al. (2019a) (11%) showed no increase in IL-8 concentration after marathon running.

Interleukin 10

Thirty-three studies were found that evaluated IL-10, about half-marathon distances (Ng et al., 2008; Cox et al., 2010; Abbasi et al., 2013; Niemelä et al., 2016), marathon (Suzuki et al., 2000, 2003; Nieman et al., 2001; Pistilli et al., 2002; Scherr et al., 2011; Nickel et al., 2012; Santos et al., 2013b, 2016; Vaisberg et al., 2013; Shanely et al., 2014; Bachi et al., 2015; Luna Junior et al., 2016; Niemelä et al., 2016; Clifford et al., 2017; Passos et al., 2019; Pugh et al., 2019; Sierra et al., 2019b; Batatinha et al., 2020; dos Santos et al., 2020; Larsen et al., 2020; Tavares-Silva et al., 2021) and ultra-marathon (Peters et al., 2001; Nieman et al., 2002, 2003, 2005, 2006, 2007; Shin and Lee, 2013; Gill et al., 2015a,b). Of this total, one study did not showed an increase in the concentration of IL-10 after marathon running (Luna Junior et al., 2016). Furthermore, the study by Santos et al. (2013b) showed a reduction after marathon running.

Tumor Necrosis Factor-α

Forty-three studies were found that evaluated TNF-α. One study show a reduction in TNF-α concentration, after marathon running (Santos et al., 2013b). Nineteen investigations, showed no differences in TNF-α immediately after half-marathon, marathon and ultra-marathon running (Drenth et al., 1995; Castell et al., 1996; Fehrenbach et al., 2000; Bonsignore et al., 2002; Suzuki et al., 2003; Nieman et al., 2006; Kim et al., 2007; Ng et al., 2008; Nickel et al., 2012; Abbasi et al., 2013; Reihmane et al., 2013; Santos et al., 2013a, 2016; Luna Junior et al., 2016; Passos et al., 2019; Sierra et al., 2019b; Batatinha et al., 2020; Costello et al., 2020; Gaggini et al., 2021). Regarding the studies that showed an increase in TNF-α levels, such results were observed after half-marathon (Bonsignore et al., 2002; Zimmer et al., 2016), marathon (Ostrowski et al., 1998, 1999; Neidhart et al., 2000; Toft et al., 2000; Nieman et al., 2001; Starkie et al., 2001; Uchakin et al., 2003; Scherr et al., 2011; Vaisberg et al., 2012; Bernecker et al., 2013; Wilhelm et al., 2014; Clifford et al., 2017; dos Santos et al., 2020; Larsen et al., 2020; Sliwicka et al., 2021; Tavares-Silva et al., 2021), ultra-marathon with variability of distances between 50 and 230 km (Nieman et al., 2007; Díaz-Castro et al., 2012; Chiu et al., 2013; Gill et al., 2015a,b; Skinner et al., 2021) and in 40 km run (Skinner et al., 2021).

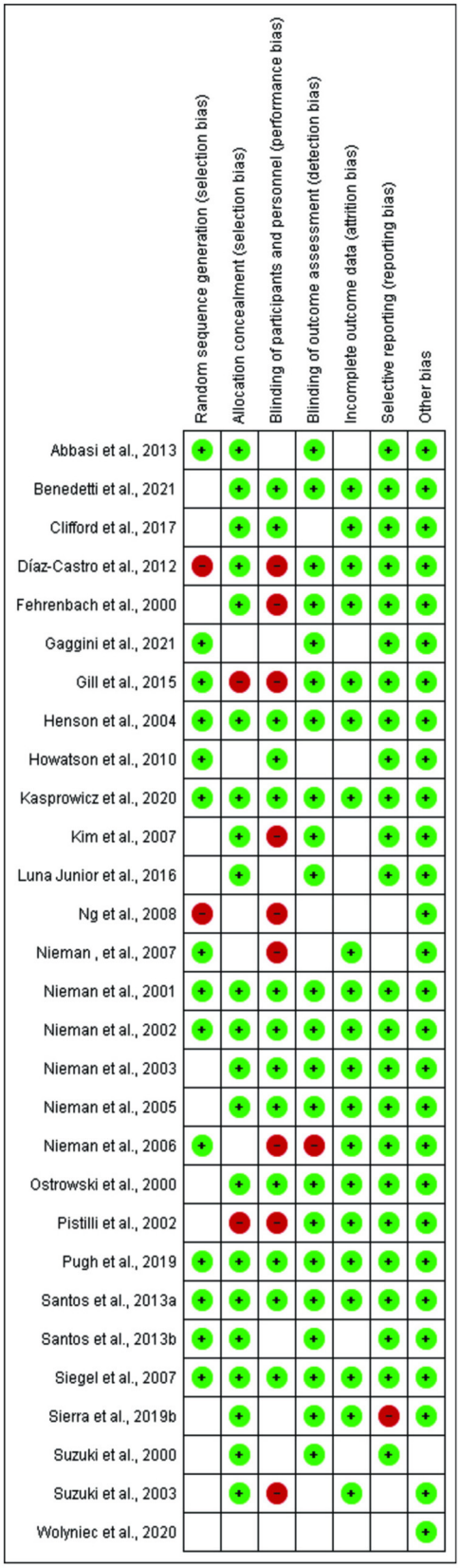


FIGURE 3 | Risk of bias summary.

Interferon Gamma

The Interferon gamma (IFN-γ) was evaluated in nine studies (Suzuki et al., 2000; Nieman et al., 2002; Henson et al., 2004; Abbasi et al., 2013; Gill et al., 2015a,b; Clifford et al., 2017; Batatinha et al., 2020; Skinner et al., 2021). There was no change in IFN-γ concentration in six studies (Suzuki et al., 2000; Abbasi et al., 2013; Gill et al., 2015b; Clifford et al., 2017; Batatinha et al., 2020; Skinner et al., 2021). In two studies, a decrease in IFN-γ concentration was found after running (Nieman et al., 2002; Henson et al., 2004), 80 km and marathon, respectively.

Leptin, Resistin, Adiponectin, and Visfatin

Five studies were found that evaluated the Leptin (Zaccaria et al., 2002; Bernecker et al., 2013; Roupas et al., 2013; Vuolteenaho et al., 2014; Sansoni et al., 2017). In the half-marathon and marathon running there were no significant changes (Zaccaria et al., 2002; Bernecker et al., 2013; Vuolteenaho et al., 2014). In the ultra-marathon running, Leptin levels reduced after the running (Zaccaria et al., 2002; Roupas et al., 2013; Sansoni et al., 2017). The Resistin was evaluated in four studies showing increased concentration after running (Roupas et al., 2013; Vuolteenaho et al., 2014; Sansoni et al., 2017; Czajkowska et al., 2020). Two studies were found that evaluated the concentration of adiponectin (Roupas et al., 2013; Vuolteenaho et al., 2014). There was an increase in adiponectin concentration after the marathon (Vuolteenaho et al., 2014). The Visfatin was evaluated in two studies (Roupas et al., 2013; Sansoni et al., 2017). Visfatin concentration increased only in the study that presented the lowest running volume 65km (Sansoni et al., 2017).

IL-12, IL-12p40, IL-12p70, IL-23, IL-33, IL-15, IL-7, IL-17a, IL-3, and IL-5

The IL-12 was evaluated in two studies: (Santos et al., 2016; Skinner et al., 2021). There was no significant difference after the run, regardless of the distance covered. Two studies evaluated the IL-12p40: (Abbasi et al., 2013; Sierra et al., 2019a). The concentration of IL-12p40 was reduced after the marathon (Sierra et al., 2019a). The IL-12p70 was also evaluated in three studies (Abbasi et al., 2013; Passos et al., 2019; Sierra et al., 2019b). There was no change in IL-12p70, regardless of running volume. Only one study evaluated the IL-23 e IL-33: (Sierra et al., 2019a), both cytokines reduced concentration after marathon (Sierra et al., 2019a). Only one study evaluated IL-15 and there was no change (Batatinha et al., 2020). IL-7 and IL-17a were evaluated in only one study (Skinner et al., 2021). Increases in the concentration of both cytokines were found after 170 km of running. Only one study evaluated IL-3 and IL-5 and there was no change (Skinner et al., 2021).

VEGF-A, Fractalkine, sTNFRII, sIL-6R, TGF-β, G-CSF, GM-CSF, MCP-1, MIP-1β, HSP72, HSP27, and HSP70

Only one study evaluated VEGF-A, finding no differences after the half-marathon, on the other hand fractalkine, showed an

TABLE 2 | The study characteristics of included studies.

Authors	Objective	Distance	No of participant	Age	Sample	Measure points[#]	Findings
Santos et al. (2013b)	To investigate effects of DHA-Rich Fish Oil Supplementation on Lymphocyte Function Before and After a Marathon race.	Marathon	21	37 ± 2	Blood	3 to 7 days before and immediately after the running.	↓ IL-2, IL-10 and TNF-α
Bernecker et al. (2013)	To investigate blood parameters of healthy men before and immediately after a marathon race.	Marathon	15	43	Blood	Directly before and immediately after the running.	↑ IL-6 and TNF-α. ↔ Leptin
Nieman et al. (2006)	To measure the influence of ibuprofen use during the 160-km Western States Endurance Run on endotoxemia, inflammation, and plasma cytokines.	160 km	30	46 ± 2	Blood	The morning before the running and 10 to 15 min after the running.	↑ IL-6, IL-1ra, IL-8, IL-10, G-CSF, MCP-1 and MIP-1β. ↔ TNF-α
Shin and Lee (2013)	The aim of this study was to assess leukocyte chemotactic cytokine and leukocyte subset responses during ultra-marathon running.	308 km	60	52 ± 5	Blood	Before and immediately after the running.	↑ IL-6 and IL-10
Ng et al. (2008)	This study investigated changes in plasma LPS concentration and immune responses (leukocyte subsets and cytokines) during a half-marathon race in warm and humid conditions.	Half-marathon	32	25 ± 3	Blood	Before and immediately after the running.	↑ IL-6, IL-10 and IL-1ra; ↔ TNF-α and IL-1β
Nieman et al. (2007)	The purpose of this study was to measure the influence of quercetin on plasma cytokines, leukocyte cytokine mRNA, and related variables in ultramarathoners competing in the 160-km Western States Endurance Run.	160 km	63	44 ± 2	Blood	The morning before the test and 10 to 15 min after the running.	↑ IL-6, IL-1ra, IL-8, IL-10, TNF-α, G-CSF, MCP-1 and MIP-1β
Roupas et al. (2013)	To evaluate the effect of prolonged intensive aerobic exercise and acute energy deficit (ultra-marathon endurance race of 180 km distance) on serum leptin, adiponectin, resistin and visfatin levels.	180 km	17	51 ± 6	Blood	Running morning, post-running and 17 to 22 h after the running.	↑ Resistin; ↔ Adiponectin and Visfatin; ↓ Leptin
Niess et al. (1999)	We supposed that the down-regulation of the baseline concentration of HO-1 in athletes reflects an adaptional mechanism to regular exercise training.	Half-marathon	10	-	Blood	Before, immediately, 3 h and 24 h after the running.	↑ IL-8 immediately and then the values returned to baseline.
Sliwicka et al. (2021)	The aim of this study was to evaluate the effects of a marathon race on selected myokines and sclerostin in 10 male recreational runners.	Marathon	10	41 ± 7	Blood	24 h before, 24 and 72 h after the running.	↑ IL-6 and TNF-α
Ostrowski et al. (2000)	The present study included data from three marathon races to investigate the hypothesis that a relationship exists between running intensity and elevated concentrations of interleukin (IL)-6 in plasma.	Marathon	53	30	Blood	One week before, immediately, 1.5 h and 3 h after the running.	↑ IL-6 and IL-1rα at 1.5 h and then the values returned to baseline.
Zaccaria et al. (2002)	With the aim of clarifying the relationship between the level of EE and the reduction in leptin levels.	Half-marathon (HM) and 100 km	HM: 23 and 100 km: 11	HM: 44 ± 2; 100 km: 46 ± 3	Blood	Immediately before and immediately after the running.	HM: ↔ Leptin 100 km: ↓ Leptin
Czajkowska et al. (2020)	To evaluate the effect of continuous, prolonged, moderate-intensity running exercise, such as running a 100 km ultra-marathon, and acute energy deficit on serum levels of pro-inflammatory adipokines: resistin and chemerin.	100 km	15	42 ± 8	Blood	Before and after the running.	↑ Resistin; ↔ Chemerin

(Continued)

TABLE 2 | Continued

Authors	Objective	Distance	No of participant	Age	Sample	Measure points[#]	Findings
Vuolteenaho et al. (2014)	The aim of the present study was to investigate the effects of marathon running on the levels of adipokines adiponectin, leptin and resistin, as well as on markers associated with cartilage degradation in inflammatory arthritis and osteoarthritis.	Marathon	46	40 ± 9	Blood	1 day before and immediately after the running.	↑ Resistina and Adiponectin; ↔ Leptin
Vaisberg et al. (2013)	The aim of this study was to evaluate the immune response elicited by exhaustive exercise in different compartments, namely, the local (upper airway mucosa) and systemic (serum) compartments, by comparing athletes that presented or not with symptoms of upper airway disease after completing a marathon.	Marathon	22	41 ± 9	Blood (B) and nasal (N)	Before, immediately and 72 h after the running.	S: ↑ IL-6 and IL-10; N: ↑ IL-6; N: ↔ IL-10
Kim et al. (2007)	The present study evaluated muscle and cartilage biomarkers, and cytokine concentration during a 200 km running event.	200 km	54	45 ± 5	Blood	10–12 h before, immediately after the 100 km and at the end of the 200 km.	↑ IL-6; ↔ TNF-α
Starkie et al. (2001)	To investigate whether prolonged, strenuous running affects the ability of circulating monocytes to produce cytokines upon stimulation and whether spontaneous cytokine production is responsible, in part, for the increased plasma cytokine concentration.	Marathon	5	-	Blood	1 h before the running, immediately, 2 h and 24 h after the running.	↑ IL-6 at all periods after the running and TNF-α only at 2 h and then the values returned to baseline.
Gaggini et al. (2021)	Evaluate the changes in plasma levels of these bioactive lipids in healthy runners performing a half-marathon, at the end of the race and after 24 h recovery, and their associations with new recently proposed and common biomarkers of immune activation.	Half-marathon	13	47 ± 6	Blood	1 day before, immediately and 24 h after the running.	↑ IL-6 and fractalkine only immediately and then the values returned to baseline. ↔ TNF-α and VEGF-A
Castell et al. (1996)	The present study investigated white blood cell numbers, together with the plasma concentrations of some amino acids, cytokines and some acute phase response markers in athletes after two separate marathon races	Marathon	38	20-40	Blood	30 min before, 15 min, 1 h and 16 h after the running.	↑ IL-6 only immediately and 1 h and IL-2 only 16 h after the running. ↔ IL-2 only immediately and 1 h and IL-1α and TNF-α at all periods after the running.
Drenth et al. (1995)	Investigated whether a 6 h endurance race such as binding plasma cytokine changes and lipopolysaccharides (LPS) stimulated ex vivo cytokine production in a whole blood culture of 19 well-trained athletes.	51–86 km	71	43 ± 8	Blood	∼18 h before and immediately after the running.	↑ IL-6 and IL-1ra; ↔ IL-1β and TNF-α
dos Santos et al. (2020)	To evaluate the prevalence of EIB in a group of recreational marathon runners without asthma, as well as to investigate both systemic and upper airway inflammatory responses and their correlation with marathon performance.	Marathon	38	38 [33-44]	Blood	24 h before and immediately after the running.	↑ IL-6, IL-8, IL-10 and TNF-α; ↔ IL-1β and IL-4
Uchakin et al. (2003)	To investigate the effects of marathon-associated stressors on cell-mediated versus humoral and anti-inflammatory versus pro-inflammatory balance, as well as their correlations with neuroendocrine response.	Marathon	15	39	Blood	24 h before, immediately, 1 h, 24 h, 48 h, 5 days and 8 days after the running.	↑ IL-6 only immediately and 1 h and TNF-α only immediately; ↔ IL-1β

(Continued)

TABLE 2 | Continued

Authors	Objective	Distance	No of participant	Age	Sample	Measure points[#]	Findings
Suzuki et al. (2003)	To investigate whether cytokines and neutrophils mediate exercise-related pathogenesis, we examined their responses and possible association after exhaustive exercise.	Marathon	10	31 ± 5	Blood (B) and urine (U)	1 day before and 10 min after the running.	B: ↑ IL-6, IL-8, IL-10, G-CSF, M-CSF and MCP-1; B: ↔ IL-1β, TNF-α; U: ↑ IL-6, IL-1β, IL-8, G-CSF, M-CSF and MCP-1; U: ↔ IL-10
Bonsignore et al. (2002)	Analyze whether the amount or duration of endurance exercise could modulate inflammatory and stress mediators, as well as circulating HPC counts.	Half-marathon (HM) and Marathon (Ma)	18	41 ± 13	Blood	9 ± 2 days before, immediately and ∼24 h after.	HM: ↑ IL-6, TNF-α and G-CSF only immediately and then the values returned to baseline. Ma: ↑ IL-6 and G-CSF and then the values returned to baseline. Ma: ↔ TNF-α
Neidhart et al. (2000)	Compare cytokine response with cartilage oligomeric matrix levels protein (COMP) and melanoma inhibitory activity (MIA) after marathon.	Marathon	8	-	Blood	Before the running, after 31 km, after the running, 1 hour, 1 day and 2 days after the running.	↑ IL-6 only immediately; IL-1ra and TNF-α only immediately and 1 h, then the values returned to baseline. ↔ IL-1β, sTNFRII and sIL-6R
Fehrenbach et al. (2000)	To verify whether the regulation of basal HSP expression in immunocompetent cells exhibits adaptation due to regular endurance training.	Half-marathon	12	32 ± 9	Blood	24 h before, immediately, 3 h and 24 h after.	↑ IL-8 only immediately and then the values returned to baseline. ↔ TNF-α
Suzuki et al. (2000)	Investigate the mechanisms of exercise-induced immune perturbations.	Marathon	16	21–39	Blood	24 h before and after the running.	↑ IL-6, IL-1ra, IL-8, IL-10 and G-CSF; ↔ IL-1β, IL-2, IL-4 and IFN-γ
Sierra et al. (2019a)	Aimed at investigating whether marathon causes cardiac fatigue and, if it is the case, whether cardiac fatigue correlates with pulmonary levels of eNO and pulmonary inflammation.	Marathon	31	39 ± 9	Sputum	24 h before and immediately after the running.	↔ IL-6, IL-8; ↓ IL-12p40, IL-23 and IL-33
Clifford et al. (2017)	Examine whether beetroot juice (BTJ) would alleviate inflammation and muscle damage after a marathon.	Marathon	34	39 ± 12	Blood	Before, after, 24 and 48 h after the running.	↑ IL-6 only immediately and 24 h, IL-1β, IL-8 e IL-10, IFN-y and TNF-α only immediately, then the values returned to baseline. ↔ IL-1ra, IL-2, MCP-1 and IL-4
Santos et al. (2016)	To determine whether running a marathon race affects neutrophil function and to characterize the underlying mechanisms.	Marathon	23	34 ± 6	Blood	24 h before, immediately, 24 h and 72 h after the running.	↑ IL-6 and IL-8 only immediately and 24 h; IL-10 only immediately and then the values returned to baseline. ↔ IL-1β, IL-12 and TNF-α
Niemelä et al. (2016)	In order to shed more light on immune system function in response to acute exercise episodes, we compared pre- and post-race values of conventional and new biomarkers of immune activation, including suPAR, CD163, pro-inflammatory (IL−6, IL-8, tumor necrosis factor-α [TNF-α]), anti-inflammatory (IL-10, growth factor-β [TGF-β]), cytokines and muscle, cardiac, renal and hepatic status markers, among typical casual long-distance running event participants.	Half-marathon (HM) and Marathon (Ma)	HM: 4; Ma: 4	HM: 39 ± 13; Ma: 26 ± 15	Blood	24 h before, 3 h and 48 h after the running.	HM and Ma: ↑ IL-6, IL-8 and IL-10 only 3 h and then the values returned to baseline. ↔ TNF-α and TGF-β
Luna Junior et al. (2016)	Was to evaluate if there is some relation between RE and cytokine production in amateur marathon runners.	Marathon	22	34 ± 6	Blood	24 h before, immediately and 72 h after.	↔ IL-6, IL-1β, IL-4, IL-8, IL-10 and TNF-α at all periods after the running.
Shanely et al. (2014)	To measure the influence of RR supplementation on exercise-induced muscle damage, delayed onset of muscle pain (DMIT), plasma cytokines, and extracellular HSP72 (eHSP72) in experienced runners completing a marathon.	Marathon	48	43 ± 1	Blood	24 h before, immediately and 1,5 h after the Marathon.	↑ IL-8, MCP-1, IL-10, IL-6, G-CSF and eHSP72 at all periods after the running.

(Continued)

TABLE 2 | Continued

Authors	Objective	Distance	No of participant	Age	Sample	Measure points[#]	Findings
Abbasi et al. (2013)	Was studied the ability of blood cultures to produce cytokines in response to endotoxin (LPS) in athletes before, 30 min after, 3 h after and 24 h after a half-marathon.	Half-marathon	16 (8M; 8F)	34 ± 9 / 38 ± 5	Blood	Before, 30 min, 3 and 24 h after the running.	M: ↑ IL-6, MCP-1 and TGF-β only 30 min, IL-1ra and IL-8 only 30 min and 3 h and IL-10 at all periods after the running. ↔ IL-12p40, IL-12p70, TNF-α and IFN-y at all periods after the running. F: ↑ IL-6, IL-8 and MCP-1 only 30 min, IL-1ra only 3 h, IL-10 only 30 min and 3 h. F: ↔ IL-12p40, IL-12p70, TNF-α, IFN-y and TGF-β at all periods after the running.
Howatson et al. (2010)	The purpose of this study was to examine the effect of a tart cherry juice blend taken before and following running a Marathon on markers of muscle damage, inflammation, and oxidative stress.	Marathon	20	38 ± 5	Blood	24 h before, immediately, 24 and 48 h after the running.	↑ IL-6 only immediately and then the values returned to baseline.
Vaisberg et al. (2012)	Investigated the effects of acute exhaustive exercise on lipid transfer to HDL.	Marathon	14	38 ± 7	Urine	Before, immediately and 72 h after the running.	↑ IL-6 and TNF-α only immediately and then the values returned to baseline.
Costello et al. (2020)	Was to examine the effect of NZBC extract supplementation taken before and following running a half-marathon race on markers of EIMD.	Half-marathon	20	29 ± 7	Blood	Before, immediately, 24 and 48 h after the running.	↔ IL-6 at all periods after the running.
Cox et al. (2010)	To investigate the effectiveness of Difflam in alleviating post-race inflammatory responses and URS in trained runners competing in a half marathon.	Half-marathon	20	35 ± 8	Blood	24 h before and immediately after the running.	↑ IL-6, IL-1ra, IL-8 and IL-10
Peters et al. (2001)	To evaluate the effects of vitamin C supplementation on changes in circulating concentrations of cortisol, adrenaline, interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1Ra) that accompany the running in the ultramarathon were measured by immuno- chemiluminescence, radioimmunoassay and ELISA procedures.	90 km	29	39 ± 7	Blood	24 h before, immediately, 24 and 48 h after the running.	↑ IL-1ra and IL-10 only immediately and then the values returned to baseline.
Sierra et al. (2019b)	Was to determine the extent of association between the AGT Met235Thr, ACE I/D and BDKRB2 +9/−9 polymorphisms with inflammation, myocardial and muscle injury, induced by endurance exercise.	Marathon	81	39 ± 1	Blood	24 h before, immediately, 24, 72 h and 15 days after the running.	↑ IL-6, IL-1β, IL-8 and IL-10 only immediately and then the values returned to baseline. ↔ TNF-α and IL-12p70 at all periods after the running.
Wołyniec et al. (2020)	Investigate post-exercise proteinuria (PEP) after long exercise - marathon and ultramarathon races.	100 km	17	40 ± 4	Blood	Immediately before and after the running.	↑ IL-6
Pugh et al. (2019)	To evaluate the effects of probiotic supplementation on gastrointestinal (GI) symptoms, circulatory markers of GI permeability, damage and immune response markers during a marathon.	Marathon	24	36 ± 7	Blood	Before and immediately after the running.	↑ IL-6, IL-8 and IL-10
Scherr et al. (2011)	We investigated the kinetics of specific cardiac biomarkers (h-FABP, hs-cTnT, NT-proBNP), inflammatory markers (interleukin-10 (IL-10), IL-6, high-sensitive C-reactive protein (hs-CRP), and TNF-α), and a marker of renal dysfunction (cystatin C) before and up to 72 h after a marathon race in a large cohort of otherwise healthy individuals.	Marathon	102	42 ± 9	Blood	One week before, immediately, 24 and 72 h after.	↑ IL-6 and TNF-α only immediately and 24 h and IL-10 at all periods after the running.

(Continued)

TABLE 2 | Continued

Authors	Objective	Distance	No of participant	Age	Sample	Measure points[#]	Findings
Passos et al. (2019)	The present study was to investigate the association between quantity of macronutrient and micronutrient daily intake and inflammation induced by long-distance exercise.	Marathon	44	41 ± 1	Blood	24 h before, immediately, 24 and 48 h after the running.	↑ IL-6, IL-1β, IL-8 and IL-10 only immediately and then the values returned to baseline. ↔ IL-12p70 and TNF-α at all periods after the running.
Yargic et al. (2019)	This study is to determine serum levels of these molecules in runners after a long-distance trail run.	35 km	37	38 ± 10	Blood	24 h before and immediately after.	↑ IL-6, IL-15 and HSP72
Mastaloudis et al. (2004)	The present study was to determine whether exercise-induced lipid peroxidation and inflammation could be alleviated by 6 weeks of prior supple-mentation with vitamins E and C in recreationally trained women and men participating in an ultramarathon run.	50 km	22	39 ± 2	Blood	1 h before, immediately, 24, 48 and 72 h after the running.	↑ IL-6 only immediately and then the values returned to baseline.
Sansoni et al. (2017)	This study was to investigate and characterize the metabolic profile (in terms of hormones involved in energy metabolism), the metabolic inflammatory profile (in terms of adipokines), and the bone metabolism by comparing the OC-mediated response in experienced MUM runners, before and after a competition, with that of control subjects with a low PA profile.	65 km	17	38 ± 7	Blood	1 h before and immediately after the running.	↑ Visfatin and Resistin. ↓ Leptin
Miles et al. (2006)	This investigation was to determine whether attenuation of the IL-6 response to strenuous endurance exercise associated with exercise-induced muscle damage occurs in higher compared to lower *ad libitum* intake of carbohydrate.	35,2 km	32	Low CHO: 42 ± 15; high CHO: 33 ± 10	Blood	1 h before, immediately, 4 and 24 h after the running.	low CHO and high CHO: ↑ IL-6 only immediately and 24 h, then the values returned to baseline.
Santos et al. (2013a)	This study was to investigate the changes in lymphocyte and neutrophil selected functions before and after a marathon race.	Marathon	15	35 ± 3	Blood	3–7 days before and immediately after the running.	↑ IL-6 and IL-1ra; ↔ TNF-α
Gill et al. (2015b)	The study aimed to determine the circulatory endotoxin concentration and cytokine profile of ultra-endurance runners (UER, $n = 19$) and a control group (CON, $n = 12$) during a five stage 230 km ultra-marathon.	230 km	19	H: 41 ± 8; M: 49 ± 4	Blood	1 h before and immediately after the running.	↑ IL-6, IL-1ra, IL-1β, IL-10, TNF-α and IFN-γ
Díaz-Castro et al. (2012)	The present study was to determine for the first time and simultaneously whether oral CoQ10 supplementation may be efficient ameliorating the oxidative stress and pro-inflammatory effects induced by the strenuous exercise.	50 km	10	39 ± 2	Blood	Immediately before and immediately after the running.	↑ IL-6, IL-1ra and TNF-α
Nieman et al. (2001)	This study was to investigate the influence of carbohydrate, gender, and age on cytokine changes in a large group of runners after two competitive marathon races.	Marathon	50	42 ± 1	Blood	Immediately before and immediately and 1,5 h after the running.	↑ IL-6, IL-1ra, IL-1β, IL-10 and TNF-α at all periods after the running.
Skinner et al. (2021)	This study was to describe and compare the effects of a trail (40 km) race and an ultra-trail (171 km) race on leukocyte concentrations and cytokine profiles.	40 km and 171 km	40 km: 11 and 171 km: 12	40 km: 37 ± 9; 171 km: 38 ± 6	Blood	Immediately before and immediately after the running.	40 km: ↑ IL-6, IL-8 and TNF-α 40 km: ↔ IL-1β, MIP-1β, MCP-1, IL-2, IFN-γ, IL-4, IL-7, IL-17a, IL-3, IL-5, IL-12 and GM-CSF 171 km: ↑ IL-6, IL-1β, MCP-1, IL-8, MIP-1β, IL-4, IL-7, IL-17a and TNF-α; 171 km: ↔ IFN-γ, IL-3, IL-2, IL-5, IL-12 and GM-CSF

(Continued)

TABLE 2 | Continued

Authors	Objective	Distance	No of participant	Age	Sample	Measure points[#]	Findings
Larsen et al. (2020)	Acute and adaptive changes in systemic markers of oxidatively generated nucleic acid modifications [i.e., 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo)] as well as inflammatory cytokines (i.e., C-reactive protein, interleukin-6, interleukin-10, and tumor necrosis factor alpha), a liver hormone [i.e., fibroblast growth factor 21 (FGF21)], and bone metabolism markers (sclerostin, osteocalcin, C-terminal telopeptide, and N-terminal propeptide of type 1 procollagen) were investigated following a marathon in 20 study participants.	Marathon	20	29 [24–37]	Blood	3–7 days before and immediately after the running.	↑ IL-6, IL-10 and TNF-α
Donnikov et al. (2009)	We studied changes in the levels of IL-6, LIF, and SCF during long exercise.	51–81 km	6		Blood	24 h before and immediately after the running.	↑ IL-6
Tavares-Silva et al. (2021)	Was to verify the effects of probiotic supplementation on cytokine production by monocytes and infections in the upper respiratory tract after an acute strenuous exercise.	Marathon	7	38 ± 3	Blood	24 h before, immediately and 1 h after the running.	↑ IL-10 only immediately and TNF-α at all periods after the running. ↔ IL-2 and IL-4 at all periods after the running.
Ostrowski et al. (1998)	Was performed to test the hypothesis that the cytokine response is locally produced in response to mechanically damaged myofibres or disrupted connective tissue in the muscle, and that a local cytokine response initiates the systemic inflammatory response.	Marathon	16	30 ± 1	Blood	1 week before, immediately and 2 h after the running.	↑ IL-6, IL-1ra and TNF-α at all periods after the running and IL-1β only immediately, then the values returned to baseline.
Bekos et al. (2019)	This study was to investigate the incidence of EIB in non-asthmatic non-professional runners and to study the association of EIB and changes in cytokine concentrations, skin or core temperature.	Half-marathon (HM) and Marathon (Ma)	HM: 36; Ma: 34	HM:36 ± 7; Ma:36 ± 7	Blood	24 h before and immediately after the running.	HM and Ma: ↑ HSP70 and HSP27
Siegel et al. (2007)	Exercise-associated hyponatremia (EAH), as defined by a blood sodium concentration [Na+] <135 mmol/L, may lead to hypotonic encephalopathy with fatal cerebral edema. Understanding the pathogenetic role of antidiuresis may lead to improved strategies for prevention and treatment.	Marathon	33	49 ± 10	Blood	24 h before, 2 and 24 h after the running.	↑ IL-6 only 2 h and then the values returned to baseline.
Nieman et al. (2003)	Changes in immune and oxidative stress parameters were measured in ultramarathon runners competing in the 160-km Western States Endurance Run.	160 km	45	46 ± 1	Blood	24 h before and immediately after the running.	↑ IL-6, IL-1ra, IL-8 and IL-10
Zimmer et al. (2016)	Investigates the short-term effects of a half marathon on immune cell proportions, pro-inflammatory cytokine levels, and recovery behavior of patients with breast cancer in the after race compared to healthy controls.	Half-marathon	9	47 ± 5	Blood	Immediately before, immediately and 24 h after the running.	↑ IL-6 only immediately and TNF-α only immediately and then the values decreased from the baseline.
Reihmane et al. (2013)	To test whether there were relations between endurance exercise-induced changes in the afore-mentioned mediators.	Half-marathon (HM) and Marathon (Ma)	HM: 22 and Ma: 18	HM: 26 ± 5; Ma: 27 ± 5	Blood	2 days before, immediately and 28 h after the running.	HM: ↔ IL-6 and TNF-α; Ma: ↑ IL-6 only immediately and then the values returned to baseline.

(Continued)

TABLE 2 | Continued

Authors	Objective	Distance	No of participant	Age	Sample	Measure points[#]	Findings
Wilhelm et al. (2014)	We measured SAPWD as a surrogate for atrial conduction and remodeling in healthy runners before and after a strenuous mountain marathon.	Marathon	10	34 ± 4	Blood	24 h before, immediately and 24 h after the running.	↑ IL-6 and TNF-α only immediately and then the values returned to baseline.
Chiu et al. (2013)	To measure the magnitude of serological response in ultra-mara-thon runners, compare the liver function tests, muscle damage markers and oxidative stress cytokines of athletes.	100 km	18	46 ± 9	Blood	1 week before, immediately and 24 h after the running.	↑ IL-6 and TNF-α all periods after the running.
Pistilli et al. (2002)	To examine the effects of a competitive marathon race on immune alterations in a relatively large group of younger and older runners.	Marathon	Older: 23 Young:75	Older: 57 Young: 37	Blood	Before, immediately and 1,5 h after the running.	Older and Young: ↑ IL-6, IL-1ra, IL-8 and IL-10 all periods after the running.
Henson et al. (2004)	To verify the influence of 6% carbohydrate intake and age on PHA-induced lymphocyte proliferation and cytokine production *in vitro*.	Marathon	25	41 ± 2	Blood	Before, immediately and 1,5 h after the running.	↓ IFN-γ all periods after the running.
Nieman et al. (2002)	To measure the influence of vitamin C compared with placebo supplementation on oxidative and immune changes in ultramarathoners competing in an ultramarathon race.	80 km	13	45 ± 2	Blood	Before and immediately after the running.	↑ IL-6, IL-1ra, IL-8 and IL-10; ↓ IL-2 and IFN-γ
Nickel et al. (2012)	To assess exercise-induced alterations of circulating dendritic cell (DC) sub-populations and toll-like receptor (TLR) expression after marathon running.	Marathon	16	E: 40 ± 7; NE: 40 ± 6	Blood	2–5 days before, immediately and 24 h after the running.	E and NE: ↑ IL-6 all periods after the running and IL-10 only immediately and TNF-α only 24 h.
Nieman et al. (2005)	Test these relationships, reasoning that elevations in plasma cytokines and significant muscle damage would occur within the first few hours of this high altitude race in the Sierra Nevada Mountains, and then be maintained for 20–30 h when correlations with CPK could be tested at the end of the race.	160 km	60	45 ± 1	Blood	The morning before the running and immediately after the running.	↑ IL-6, IL-1ra, IL-8, IL-10, MCP-1, MIP-1β and G-CSF
Toft et al. (2000)	Was to investigate whether fish oil supplementation was able to modulate the acute-phase response to strenuous exercise.	Marathon	10	28 [24–43]	Blood	One week before, immediately, 1.5, and 3 h after the running.	↑ IL-6, IL-1ra and TNF-α all periods after the running. ↔ TGFβ all periods after the running.
Bachi et al. (2015)	To investigate how physical and psychological changes induced in mara-thon runners by training and by the race can affect mood states, hormones and cytokines.	Marathon	20	35 ± 9	Blood	24 h before, immediately, and 72 h after the running.	↑ IL-10 only immediately and then the values returned to baseline. ↔ IL-8 only immediately and then the values decreased from the baseline.
Scherr et al. (2012)	To determine whether ingestion of NAB polyphenols for 3 weeks before and 2 weeks after a marathon would attenuate postrace inflammation and decrease URTI incidence.	Marathon	63	42 [35–49]	Blood	1 week before, immediately, 24 and 72 h after the running.	↑ IL-6 only immediately and then the values returned to baseline.
Ostrowski et al. (1999)	Investigates to what extent and by which time course prolonged strenuous exercise influences the plasma concentration of pro-inflammatory and inflammation responsive cytokines as well as cytokine inhibitors and anti-inflammatory cytokines.	Marathon	10	28 [24–37]	Blood	1 week before, immediately and every 30 min until 4 h after the running.	↑ IL-6 and IL-1ra all periods after the running, IL-1β only immediately and 30 min and TNF-α only immediately and until 3 h after the running, then the values returned to baseline.
Batatinha et al. (2020)	To evaluate the alterations caused by a marathon in the lymphocyte population and function, and the effects of probiotics in this process.	Marathon	13	40 ± 7	Blood	24 h before and 1 h after the running.	↑ IL-6, IL-8 and IL-10; ↔ IL-2, IL-4, IL-1β, TNF-α, IFN-γ and IL-15
Benedetti et al. (2021)	Monitored for the first time in ultramarathon athletes running the 24-h competition, an extremely demanding race in terms of muscular and physiological exertion.	99–218 km	22	42 ± 11	Blood	3 h before and immediately after the running.	↑ IL-6

(Continued)

TABLE 2 | Continued

Authors	Objective	Distance	No of participant	Age	Sample	Measure points[#]	Findings
Kasprowicz et al. (2020)	To examine whether two-week high-dose supplementation (10,000 IU/day) of vitaminD3can have an influence on 25 (OH)D serum concentration, and secondly, whether it can aecthepcidin, iron, and IL-6 responses to a 100-km ultra-marathon.	100km	10	42 ± 8	Blood	Before, immediately and 12 h after the running.	↑ IL-6 only immediately and then the values returned to baseline.
Gill et al. (2015a)	To determine circulatory endotoxin concentration and cytokine profile of ultra-endurance runners in response to a 24-h continuous ultra-marathon competition conducted in temperate ambient conditions; and additionally, to determine the relationship between these responses with gastrointestinal symptoms.	122–208 km	17	40 ± 7	Blood	Before and immediately after the running.	↑ IL-6, IL-1ra, IL-1β, IL-8, IL-10 and TNF-α; ↔ IFN-γ

#, Only until 72 hours after; ↑, Significant increase; ↓, Significant decrease; ↔, no change; IL, Interleukin; IL-1ra, Interleukin-1 receptor alfa; TNF-α, tumor necrosis factor- α; IFN-γ, interferon gamma; G-CSF, granulocyte colony-stimulating factor; MCP-1, monocyte chemotactic protein 1; MIP-1β, macrophage inflammatory protein 1 beta; TGF-β, transforming growth factor-β; VEGF-A, Vascular endothelial growth factor-A; sTNFRII, tumor necrosis factor type II p75; sIL-6R, interleukin-6 receptor gp80; HSP, heat shock protein; GM-CSF, granulocyte-macrophage colony-stimulating factor; B, blood; N, nasal; U, urine; HF, half-marathon; Ma, marathon; M, male; F, female; CHO, carbohydrate.

increase in its concentration after the half-marathon (Gaggini et al., 2021). The sTNFRII and sIL-6R were evaluated in a study (Neidhart et al., 2000), there was no change after marathon. Three studies evaluated the TGF-β (Toft et al., 2000; Abbasi et al., 2013; Niemelä et al., 2016) and no significant differences were found after running. The G-CSF was evaluated in seven studies (Suzuki et al., 2000, 2003; Bonsignore et al., 2002; Nieman et al., 2005, 2006, 2007; Shanely et al., 2014). G-CSF concentration increased after running in all studies, regardless of running distance. Eight studies evaluated MCP-1 (Suzuki et al., 2003; Nieman et al., 2005, 2006, 2007; Abbasi et al., 2013; Shanely et al., 2014; Clifford et al., 2017; Skinner et al., 2021) and only in one study there was no change in the concentration of MCP-1 (Clifford et al., 2017). Four studies evaluated the MIP-1β (Nieman et al., 2005, 2006, 2007; Skinner et al., 2021) and showed concentration increase. The GM-CSF was evaluated in one study (Skinner et al., 2021) and did not showed alterations. Two studies evaluated HSP72, there was an increase in concentration after running in both studies (Shanely et al., 2014; Yargic et al., 2019). The HSP27 e HSP70 were evaluated in only one study (Bekos et al., 2019). Both showed an increase after the half-marathon and marathon.

Meta-Analysis

For the meta-analysis, were included the individual and summarized effects of the studies that analyzed the IL-6 (n = 20), IL-1ra (n = 10), IL-Iβ (n = 7), IL-2 (n = 5), IL-4 (n = 3), IL-8(n = 13), IL-10 (n = 12), TNF-α (n = 11) and INF-y (n = 6). In the analysis of subgroups performed with IL-6 in consideration of half-marathon (n = 2), marathon (n = 8) and ultra-marathon (n = 10), in the pre vs post-running comparison, an increase was observed in all the distances (SMD −6.69; IC 95%: −8.14, −5.24; $p < 0.0001$) highlighted a regular rise to running distance: half-marathon (SMD −1.36; IC 95%: −1.82, −0.89, Ch^2:0.58; tau^2:0.00; $p < 0.0001$), marathon (SMD −6.81; IC 95%: −9.26, −4.37; Ch^2:481.37 tau^2:11.88; $p < 0.0001$) and ultra-marathon (SMD−8.00 IC 95%: −10.47,−5.53; Ch^2:328.40; tau^2:14.19; $p < 0.0001$). Meta-regression analysis showed no significant regressions on half marathon, marathon and ultra-marathon (p= 0.864) (**Figure 4**).

An increase in the concentration of IL-1ra (SMD−5.65; IC 95%:−7.13,-4.17; $p < 0.0001$), IL-1ra (SMD −0.95; IC 95%: −1.39,-0.50; $p < 0.0001$), IL-8 (SMD −5.38; IC 95%: −7.25,-3.51; $p < 0.0001$), IL-10 (SMD −32.59; IC 95%: −45.99,-19.19; $p < 0.0001$) and TNF-α (SMD −0.83; IC 95%: −1.00,-0.67; $p <$ 0.0001). A reduction in the concentration of IL-2 (SMD 57.74; IC 95%: 37.12, 78.36; $p < 0.0001$) and INF-y (SMD 1.97; IC 95%: 0.23,3.71; $p < 0.03$) and there was no change in the IL-4 (SMD −0.10; IC 95%: −0.42,0.23; $p < 0.56$). Evidence of heterogeneity and inconsistency was found for IL-1ra (Ch^2 = 230.31; Tau^2 =5.17; I^2 = 96%), IL-1ra (Ch^2 = 25.28; Tau^2 = 0.25; I^2 = 76%), IL-2 (Ch^2 = 374.24; Tau^2 = 464.72; I^2 = 99%), IL-8 (Ch^2 =849.11; Tau^2 = 11.46; I^2 = 99%), IL-10 (Ch^2 =550.64; Tau^2 = 5,179.66; I^2 = 96%), TNF-α (Ch^2 =274.93; Tau^2 = 2.20; I^2 = 96%) and INF-y (Ch^2 =138.99; Tau^2 = 4.38; I^2 = 96%) (**Figure 5**).

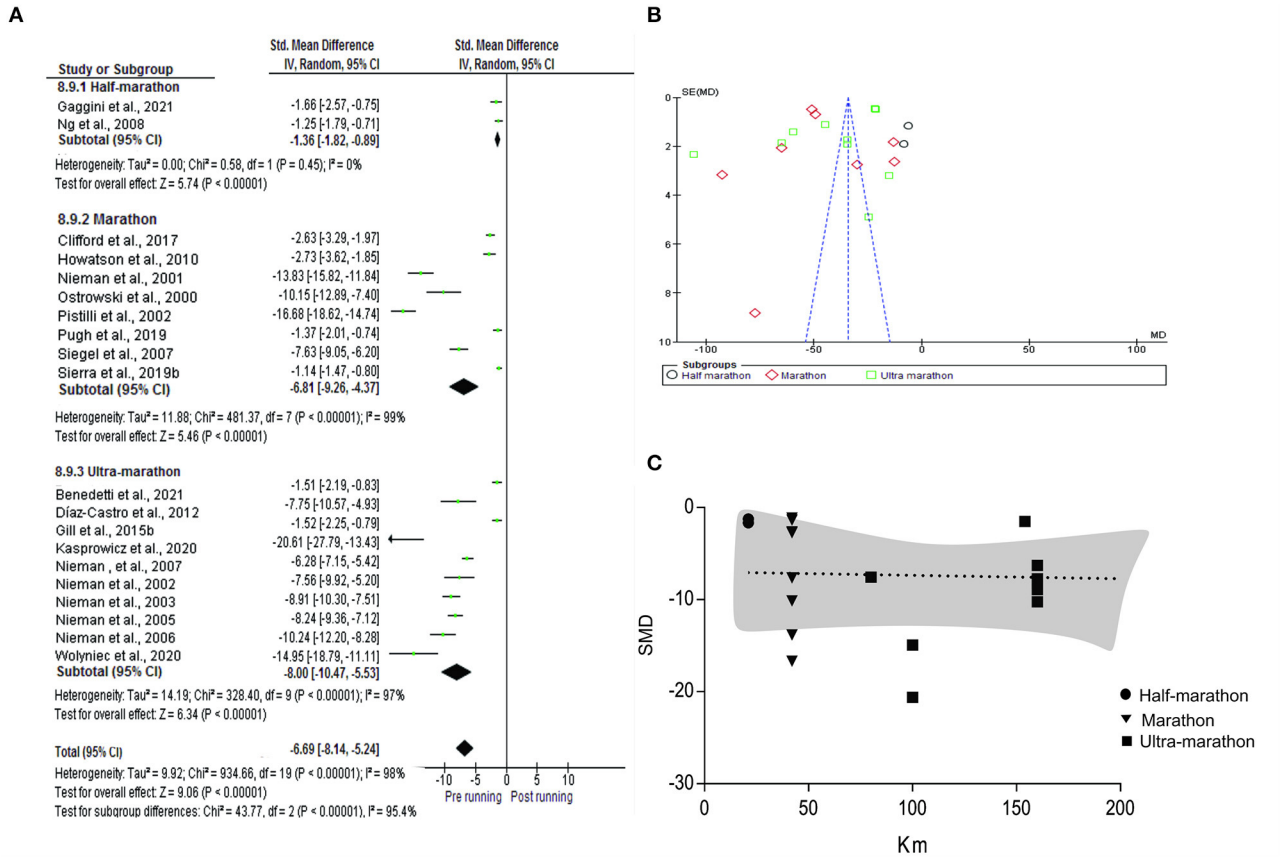


FIGURE 4 | Meta-analysis of half-marathon, marathon, and ultra-marathon. **(A)** Forest plot, **(B)** Funnel plot, and **(C)** meta-regression analyses.

DISCUSSION

In the current report, we describe changes in impacts of running volume on the concentration of cytokines in half-marathon, marathon and ultra-marathon. Was verified increase in inflammation status immediately after completing the running. The meta-analysis showed that the running volume variability modulate in the maintenance of homeostasis and cell signaling. On the other hand, the increase in volume does not proportionally increase the concentration of IL-6, IL-1ra, IL-1β, IL-8, IL-10 and TNF-α after the running. In contrast, few studies evaluated adipokines. Prolonged aerobic exercise exerts a huge impact on metabolism and energy balance, is an exhausting effort for the whole organism and leads to a pro-inflammatory profile.

Cytokines are important mediators regularizing the immune response, and their enhancement may yield valuable information pertinent to questions like transient post-running immunosuppression, beneficial anti-inflammatory. Although a high increase in cytokine concentration was expected to occur after long-distance running, a key point was if an increase in concentration proportional to running volume would occur. This fact was not confirmed when analyzing the studies that evaluated the concentration of IL-6 by subgroups: half-marathon, marathon and ultra-marathon (**Figure 3**). Regardless of if running over 42 km are more complex, results show that to achieve homeostatic equilibrium, the integrated action of the neuroendocrine and immune systems is necessary (Bachi et al., 2015). The higher serum levels of growth hormone in athletes at rest and the higher production of cytokines without previous stimulus suggests that long distance runners present mechanisms that may be associated with preparing the body to perform prolonged strenuous exercise. Therefore, the inherent vulnerability to exercise induced inflammatory alterations is passible adaptation mechanisms to training (Scherr et al., 2011).

Regardless of whether long-distance running athletes are more prepared, the studies that evaluated the recovery time of IL-6 showed after 21km, the less time required to return to baseline conditions. So, several hypotheses have been raised to explain that recovery seems to be more associated with running volume including intensity of exercise or training status (Scherr et al., 2011; Gaggini et al., 2021). However, the results are still controversial. If on the one hand, in the quantification of the concentration of IL-6 after a half-marathon, it was only verified an increase immediately after the running, occurs a return to baseline conditions after the running (Gaggini et al., 2021) or also even 30 minutes (Abbasi et al., 2013). And that pattern was different after marathon, kinetics of serum IL-6

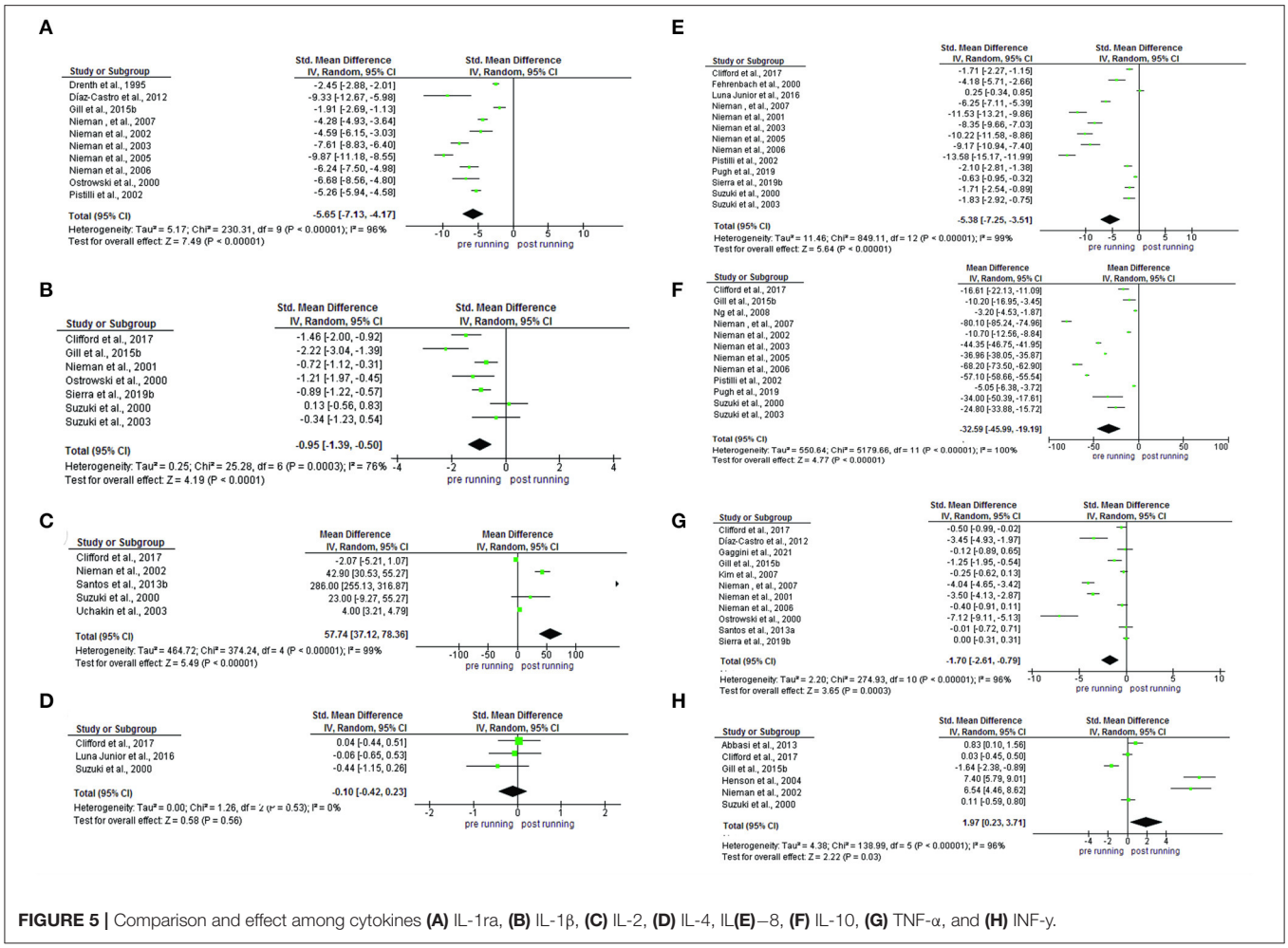


FIGURE 5 | Comparison and effect among cytokines **(A)** IL-1ra, **(B)** IL-1β, **(C)** IL-2, **(D)** IL-4, IL**(E)**–8, **(F)** IL-10, **(G)** TNF-α, and **(H)** INF-y.

concentration prolonged elevation for at least 24 h (Scherr et al., 2011). Furthermore, the studies that evaluated 100 km (Chiu et al., 2013) and 50 km (Mastaloudis et al., 2004) showed baseline conditions in 24 h.

In accordance with the reports of studies, was verified that levels of six cytokines, IL-6, IL-1ra, IL-1β, IL-8, IL-10 and TNF-α rose strongly in response to race competition. Unlike IL-2 and INF-y, whom decreased. Post running levels of IL-4, remained near pre running or at no detectable levels. This outcome corroborates with classical studies (Nieman et al., 2001). Reported long- running induced muscle cell metabolic activity and damage appear to be important triggers of macrophage and neutrophil migration and cytokine release (Terra et al., 2012). The low post running levels found in studies as IL-4 may be due to the strong inhibitory effects of IL-10, IL-1ra and IL-6 which together help prevent an overly active systemic inflammation (Suzuki et al., 2000). Several explain about the decrease in the IL-2 and INF-y reported increased lymphocyte proliferation (Santos et al., 2013b). Leukocyte function is modulated by different pathways such as proliferation control, cytokine and anti-inflammatory mediator, generation, adhesion molecule expression, and cell death (Akhtar Khan, 2010).

Physical activity increases myokine levels. The IL-6 was observed on a large scale in 62 studies. IL-6 plays a positive role in glucose metabolism (Glund and Krook, 2007; Shoghi et al., 2008; Pedersen, 2011), increasing its uptake by myocytes (Carey et al., 2006), and elevating insulin-stimulated glycogen synthesis in skeletal muscle (Weigert et al., 2005). Intramuscular concentration of IL-6 mRNA (Keller et al., 2001) and protein release (Steensberg et al., 2001) are exacerbated when intramuscular glycogen is compromised, suggesting that IL-6 functions as an energy sensor (Pedersen and Febbraio, 2008). The level of IL-6 was shown to increase exponentially proportional to the exercise duration and the amount of muscle mass involved in the exercise (Pedersen, 2016). Running, which involves several large muscle groups, is the mode of exercise where most marked increases in plasma IL-6 have been observed (Pedersen and Febbraio, 2008). Exercise duration is the most important factor in determining post-exercise plasma IL-6 amplitude (Reihmane et al., 2013). In this perspective, plasma IL-6 can increase up to 40 times after a marathon (Reihmane et al., 2013; Santos et al., 2016; Larsen et al., 2020; Skinner et al., 2021), with the elevation being equivalent to that observed in negative health status (Skinner et al., 2021).

The Inflammatory response is associated with the pro-inflammatory cytokine storm (Smith et al., 2020). This process is due in part to the activation of M1 type macrophages, which have characteristic proinflammatory such as IL-1β (Bent et al., 2018). This important cytokine is one of the mediators of inflammation and is involved in several cellular activities, including cell proliferation, differentiation, and apoptosis (Conti et al., 2002; Bent et al., 2018). The present review identified variability in the effects of running volume on IL-1β levels. The majority demonstrated significant increase in long-distance runners. Thus, the increased plasma concentrations of IL-1β after the Prolonged aerobic exercise could reflect exercise-mediated inflammasome NLRP3 pathway activation during long-running caused by the extreme effort. Oxidized hemoglobin, a hemolysis related product, has been identified as a potent trigger of NLRP3 activation and IL-1β production (Skinner et al., 2021).

Interleukins 2, 4, and 8 actively participate in the structure of the immune system, in the maturation of T lymphocytes, activation of macrophages *via* alternative pathways and migration of neutrophils, respectively. Few studies have evaluated the responses caused by running volume in IL-2 and 4. Only two studies identified a reduction in IL-2 and INF-y levels in marathon runners, studies showed that low levels of this protein are associated with impairment of immunological memory and character pathologies inflammatory including rheumatoid arthritis (Arenas-Ramirez et al., 2015; Wu et al., 2020). In a previous study, was reported a significant reduction of IL-2 and INF-y production in runners at the completion of an 80 km ultra-marathon (Nieman et al., 2002). In addition, other exhaustive aerobic exercise depression of IL-2 and INF-y 1 h after the event (Weinstock et al., 1997; Nieman et al., 2005) and after and 1.5 h after completion of the marathon race (Henson et al., 2004). These findings are consistent with our data showing a significant decline in both induced production of INF-y (unadjusted and adjusted per T-cell) and IL-2. Additionally, in IL-4 levels, no differences were found in runners. Inhibitory cytokines, namely IL-4 have been shown to upregulate IL-1ra production and downregulate IL-1β and TNF-α (Suzuki et al., 2000). Cortisol concentration also increased after the race, and it has been shown that it can also inhibit the production of several cytokines (Nieman et al., 2001).

Most of the thirteen studies used in our systematic review demonstrated high levels of IL-8 in the blood of half-marathon, marathon, and 160 km runners. Studies demonstrate the effective participation of physical activity and physical exercise, especially the aerobic emphasis on the modulation of this myokine (Barbalho et al., 2020). IL-8 is a classical proinflammatory cytokine, it was originally identified as a chemoattractant factor for neutrophils (dos Santos et al., 2020). Beyond its proinflammatory role, IL-8 also presents a prominent angiogenic function and, as mentioned, is considered a myokine (dos Santos et al., 2020). According to the literature, the increased IL-8 levels in response to muscle damage induced by a physical exercise session are mainly associated with the regulation of muscle angiogenesis by its binding to the CXC receptor 2 (CXCR2) expressed in microvascular endothelial cells in order to improve muscle regeneration (Frydelund-Larsen et al., 2007).

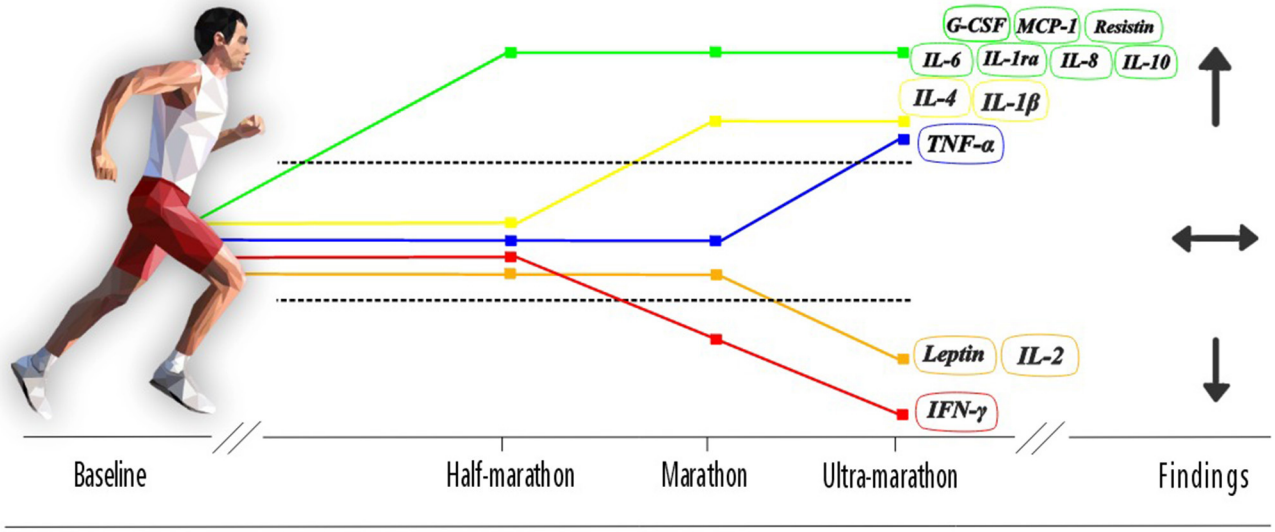
The IL-10 and TNF-α carry out antagonistic immune responses. Biologically, IL-10 works by deactivating macrophages to produce inhibitory effects on Natural Killer (NK) cells and T lymphocytes, moreover, to playing a fundamental anti-inflammatory role (Saraiva and O'Garra, 2010). Our data reveal that different running volumes significantly elevated blood and urine IL-10 levels. Pedersen (2017) demonstrated that physical exercise can potentiate IL-10 activity, reducing inflammation that is part of the synthesis of several cardiometabolic pathologies, including type 2 diabetes mellitus and cardiovascular diseases. This points to the possible preventive character of this immunological marker in these conditions (Pedersen, 2017).

On the other hand, TNF-α is identified as one of the considerable cytokines for inflammation in different organisms. Furthermore, it is linked to the emergence and development of different types of cancers (Balkwill, 2009). In the data used in this review, we found a variety of responses within the production of this protein after different running volumes. Furthermore, it is known that acute physical exercises that require high demands of metabolic effort, including ultra-marathon, can promote an increase in this cytokine (Uchida et al., 2014). Results indicate that during a marathon run, the pro-inflammatory markers TNF-α are not produced by blood mononuclear cells on the mRNA level to a clinically relevant extent during a marathon running (Bernecker et al., 2013). There is, however, a significant increase in TNF-α in the plasma, suggesting a local production or release from the stressed skeletal muscle tissues of these cytokines. More studies are needed in high running volumes for better understand (Uchida et al., 2014).

Were also observed changes caused by high running volume in leptin, resistin, adiponectin and visfatin levels. Leptin participates in the regulation of food intake, energy balance and reproductive system (Zhang and Chua, 2017). Furthermore, its deficiency in its production is related to the genesis of obesity. Only two studies looked at the possible effects of running volume on the production of this hormone in the blood. Where only, Zaccaria et al. (2002) observed a significant reduction in leptin after 100 km of running. Several studies with different experimental models demonstrate that aerobic exercise can bring changes in leptin levels, increasing its sensitivity and acting preventively on obesity (Yetgin et al., 2018; Fernandes et al., 2020).

In contrast, high levels of resistin were found in the blood of marathon runners and 100 km away. This protein is secreted by adipose tissue, immune and epithelial cells in mammals, it has the function of blocking the action of leptin, reducing satiety (Acquarone et al., 2019). Moreover, clinical studies have shown that the increase in resistin after physical exercise is associated with a reduction in fatigue, playing a fundamental role in the reduction of musculoskeletal and joint inflammatory events, common in pathologies such as fibromyalgia, obesity, and high demands for effort physical and running volumes (Bjersing et al., 2013).

Adiponectin's are proteins produced mainly because they play a fundamental role in glycemic and free fatty acid control. In our data, an increase in adiponectin levels in marathon runners was observed only in one study. However, the role of physical



The behavior of plasma cytokine concentration in runs half-marathon, marathon, and ultramarathon. Notes: ■, ■, ■, ■ and ■: Post-running only on period immediately after half-marathon, marathon or ultra-marathon; ↑, Significant increase; ↓, Significant decrease; ↔, no change; IL, Interleukin; IL1ra, Interleukin-1 Receptor Alfa; TNF-α, Tumor Necrosis Factor-Alfa; IFN-γ, Interferon Gamma; G-CSF, Granulocyte Colony-Stimulating Factor; MCP-1, Monocyte Chemotactic Protein 1; MIP-1β, Macrophage Inflammatory Protein 1 Beta. Pngwing. (2021).

FIGURE 6 | Changes in cytokines concentration following long-distance running (Pngwing, 2021).

exercise in different modalities and protocols in the increase of adiponectin's is well established (Li et al., 2019). Finally, visfatin, it is produced by visceral adipose tissue with insulinomimetic activities with systemic and local action. Within the studies that evaluated this biomarker, no significant differences were observed. However, its role associated with physical exercise needs to be elucidated (Jamurtas et al., 2015).

In addition, we also consider other limitations that have been little explored in the research, mainly related to the analysis between sex, ultra-marathon distance and follow-up of 24, 48, and 72 h. Only 1 study discriminated the results differentiating by sex. Although 31% of studies evaluated 24-h follow-up, only 3% was evaluated in ultra-marathon running, of these, 48 and 72-h follow-up was evaluated in 2 and 1 studies, respectively. There was great variability of distance between the ultra-marathon running (51–308 km) generating high amplitude (6:00 to 62:20 h) between the times of conclusion of the races. All these facts, added to the heterogeneity, made it difficult to carry out a quantitative analysis, suggesting the expansion of new studies contemplating these objectives, as well as, differentiating single and multi-day ultra-marathons.

In conclusion, the trans-signaling of cytokines results in inflammation and is therefore linked to high-grade inflammatory. Long-distance running promote an increase in the concentration of IL-6, IL-1ra, IL-1β, IL-8, IL-10 and TNF-α and a decrease in the concentration of IL-2 (**Figure 6**). The effects of an acute bout of prolonged aerobic exercise will protect against chronic systemic inflammation. The time to return to baseline values showed a substantial and dose-dependent relationship with run volume. The concentration of IL-6 was robustly studied in long-distance running present in more than 80% of the selected articles and the marathon running was the most explored. Further studies with adipokines are recommended mainly in ultra-marathons as well, and further investigations related to runner level, age, sex, follow-up, multi-stage ultra-marathons also suggested.

In practical considerations, the ability of a runner to minimize the effects of an acute bout of prolonged aerobic exercise will against chronic systemic inflammation may be identified as one of the determinant factors of performance. The athletes attention must be taken in age regulations, nutrition, training status and race-specific factors (elevation change, such as distance, level of medical assistance, ambient temperatures and type of provisions provided by the race organizers), being able decrease induced physical and physiological distresses and speeding up recovery and rehabilitation from injuries. Long-distance running may have different physiological requirements for ultra-marathon, marathon, and half-marathon. Consequently the strategies of runners provide a challenge for inflammatory process, and there is a general interest in interpret its alterations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

MA, DS, and RS: conceptualization. MA, DS, EP, and DO: methodology. RS, MS, DP, MA, and DS: writing–original draft. FA, DS, and LV-S: formal analysis. RS, MA, DS, EP, DO, MS,

and FA: writing-review and editing. All authors contributed to the final version of the manuscript.

FUNDING

We would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES, Finance Code 001 and CAPES/PROCAD-2013).

ACKNOWLEDGMENTS

We would like to thank the Coordination of the Post-Graduation Program of Physical Education, Federal University of Sergipe (UFS), the members of the Group of Studies and Research of Performance, Sport, Health and Paralympic Sports (GEPEPS), and the members of the Running Club of UFS.

REFERENCES

Abbasi, A., Fehrenbach, E., Hauth, M., Walter, M., Hudemann, J., Wank, V., et al. (2013). Changes in spontaneous and lps-induced ex vivo cytokine production and mrna expression in male and female athletes following prolonged exhaustive exercise. *Exerc. Immunol. Rev.* 19, 8–28.

Acquarone, E., Monacelli, F., Borghi, R., Nencioni, A., and Odetti, P. (2019). Resistin: A reappraisal. *Mech. Ageing Dev.* 178, 46–63. doi: 10.1016/j.mad.2019.01.004

Akhtar Khan, N. (2010). Polyunsaturated fatty acids in the modulation of T-cell signalling. *Prostaglandins, Leukot. Essent. Fat. Acids* 82, 179–187. doi: 10.1016/j.plefa.2010.02.023

Allen, D. L., Hittel, D. S., and McPherron, A. C. (2011). Expression and function of myostatin in obesity, diabetes, and exercise adaptation. *Med. Sci. Sport. Exerc.* 43, 1828–1835. doi: 10.1249/MSS.0b013e3182178bb4

Allen, J., Sun, Y., and Woods, J. A. (2015). "Exercise and the Regulation of Inflammatory Responses," in *Progress in Molecular Biology and Translational Science* (Elsevier Inc.) p. 337–354. doi: 10.1016/bs.pmbts.2015.07.003

Arenas-Ramirez, N., Woytschak, J., and Boyman, O. (2015). Interleukin-2: biology, design and application. *Trends Immunol.* 36, 763–777. doi: 10.1016/j.it.2015.10.003

Bachi, A. L. L., Rios, F. J. O., Vaisberg, P. H. C., Martins, M., Cavalcante de, S.á, M., Victorino, A. B., et al. (2015). Neuro-immuno-endocrine modulation in marathon runners. *Neuroimmunomodulation* 22, 196–202. doi: 10.1159/000363061

Balkwill, F. (2009). Tumour necrosis factor and cancer. *Nat. Rev. Cancer* 9, 361–371. doi: 10.1038/nrc2628

Barbalho, S. M., Prado Neto, E. V., De Alvares Goulart, R., Bechara, M. D., Baisi Chagas, E. F., Audi, M., et al. (2020). Myokines: a descriptive review. *J. Sports Med. Phys. Fitness* 60, 1583–1590. doi: 10.23736/S0022-4707.20.10884-3

Batatinha, H., Tavares-Silva, E., Leite, G. S. F., Resende, A. S., Albuquerque, J. A. T., Arslanian, C., et al. (2020). Probiotic supplementation in marathonists and its impact on lymphocyte population and function after a marathon: a randomized placebo-controlled double-blind study. *Sci. Rep.* 10, 18777. doi: 10.1038/s41598-020-75464-0

Bekos, C., Zimmermann, M., Unger, L., Janik, S., Mitterbauer, A., Koller, M., et al. (2019). Exercise-induced bronchoconstriction, temperature regulation and the role of heat shock proteins in non-asthmatic recreational marathon and half-marathon runners. *Sci. Rep.* 9, 4168. doi: 10.1038/s41598-019-39983-9

Benedetti, S., Gemma Nasoni, M., Palma, F., Citarella, R., and Luchetti, F. (2021). Serum changes in sTWEAK and its scavenger receptor sCD163 in ultramarathon athletes running the 24-h race. *Cytokine* 137, 155315. doi: 10.1016/j.cyto.2020.155315

Bent, R., Moll, L., Grabbe, S., and Bros, M. (2018). Interleukin-1 beta—A friend or foe in malignancies? *Int. J. Mol. Sci.* 19, 2155. doi: 10.3390/ijms19082155

Bernecker, C., Scherr, J., Schinner, S., Braun, S., Scherbaum, W. A., and Halle, M. (2013). Evidence for an exercise induced increase of TNF-α and IL-6 in marathon runners. *Scand. J. Med. Sci. Sports* 23, 207–214. doi: 10.1111/j.1600-0838.2011.01372.x

Bjersing, J. L., Erlandsson, M., Bokarewa, M. I., and Mannerkorpi, K. (2013). Exercise and obesity in fibromyalgia: beneficial roles of IGF-1 and resistin? *Arthritis Res. Ther.* 15, R34. doi: 10.1186/ar4187

Bonsignore, M. R., Morici, G., Santoro, A., Pagano, M., Cascio, L., Bonanno, A., et al. (2002). Circulating hematopoietic progenitor cells in runners. *J. Appl. Physiol.* 93, 1691–1697. doi: 10.1152/japplphysiol.00376.2002

Carey, A. L., Steinberg, G. R., Macaulay, S. L., Thomas, W. G., Holmes, A. G., Ramm, G., et al. (2006). Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes* 55, 2688–2697. doi: 10.2337/db05-1404

Castell, L. M., Poortmans, J. R., Leclercq, R., Brasseur, M., Duchateau, J., and Newsholme, E. A. (1996). Some aspects of the acute phase response after a marathon race, and the effects of glutamine supplementation. *Eur. J. Appl. Physiol. Occup. Physiol.* 75, 47–53. doi: 10.1007/s004210050125

Cerqueira, É., Marinho, D. A., Neiva, H. P., and Lourenço, O. (2020). Inflammatory effects of high and moderate intensity exercise—a systematic review. *Front. Physiol.* 10, 1–14. doi: 10.3389/fphys.2019.01550

Chiu, Y.-H., Hou, S.-K., How, C.-K., Li, L.-H., Kao, W.-F., Yang, C.-C., et al. (2013). Influence of a 100-km ultra-marathon on hepatitis b carrier runners. *Int. J. Sports Med.* 34, 841–845. doi: 10.1055/s-0032-1331769

Clifford, T., Allerton, D. M., Brown, M. A., Harper, L., Horsburgh, S., Keane, K. M., et al. (2017). Minimal muscle damage after a marathon and no influence of beetroot juice on inflammation and recovery. *Appl. Physiol. Nutr. Metab.* 42, 263–270. doi: 10.1139/apnm-2016-0525

Conti, G., De Pol, A., Scarpini, E., Vaccina, F., De Riz, M., Baron, P., et al. (2002). Interleukin-1beta and interferon-gamma induce proliferation and apoptosis in cultured Schwann cells. *J. Neuroimmunol.* 124, 29–35. doi: 10.1016/S0165-5728(02)00003-6

Costello, R., Willems, M. E. T., Myers, S. D., Myers, F., Lewis, N. A., Lee, B. J., et al. (2020). No effect of New Zealand blackcurrant extract on recovery of muscle damage following running a half-marathon. *Int. J. Sport Nutr. Exerc. Metab.* 30, 287–294. doi: 10.1123/ijsnem.2019-0312

Cox, A. J., Gleeson, M., Pyne, D. B., Saunders, P. U., Callister, R., and Fricker, P. A. (2010). Respiratory symptoms and inflammatory responses to Difflam throat-spray intervention in half-marathon runners: A randomised controlled trial. *Br. J. Sports Med.* 44, 127–133. doi: 10.1136/bjsm.2008.048298

Czajkowska, A., Ambroszkiewicz, J., Mróz, A., Witek, K., Nowicki, D., and Małek, Ł. (2020). The effect of the ultra-marathon run at a distance of 100 kilometers on the concentration of selected adipokines in adult men. *Int. J. Environ. Res. Public Health* 17, 4289. doi: 10.3390/ijerph17124289

Das, D. K., Graham, Z. A., and Cardozo, C. P. (2020). Myokines in skeletal muscle physiology and metabolism: Recent advances and future perspectives. *Acta Physiol.* 228, 0–3. doi: 10.1111/apha.13367

de Oliveira dos Santos, A. R., de Oliveira Zanuso, B., Miola, V. F. B., Barbalho, S. M., Santos Bueno, P. C., Flato, U. A. P., et al. (2021). Adipokines, myokines, and hepatokines: crosstalk and metabolic repercussions. *Int. J. Mol. Sci.* 22, 2639. doi: 10.3390/ijms22052639

Díaz-Castro, J., Guisado, R., Kajarabille, N., García, C., Guisado, I. M., de Teresa, C., et al. (2012). Coenzyme Q10 supplementation ameliorates inflammatory signaling and oxidative stress associated with strenuous exercise. *Eur. J. Nutr.* 51, 791–799. doi: 10.1007/s00394-011-0257-5

Donnikov, A. E., Shkurnikov, M. Y., Akimov, E. B., Grebenyuk, E. S., Khaustova, S. A., Shahmatova, E. M., et al. (2009). Effect of a six-hour marathon ultra-race on the levels of IL-6, LIF, and SCF. *Bull. Exp. Biol. Med.* 148, 819–821. doi: 10.1007/s10517-010-0825-3

dos Santos, J., de, M. B., Bachi, A. L. L., Junior, L. A. L., Foster, R., Sierra, A. P. R., et al. (2020). The relationship of IL-8 and IL-10 myokines and performance in male marathon runners presenting exercise-induced bronchoconstriction. *Int. J. Environ. Res. Public Health* 17, 1–15. doi: 10.3390/ijerph17082622

Drenth, J. P. H., Van Uum, S. H. M., Van Deuren, M., Pesman, G. J., Van der Ven-Jongekrijg, J., and Van der Meer, J. W. (1995). Endurance run increases

circulating IL-6 and IL-1ra but downregulates ex vivo TNF-alpha and IL-1 beta production. *J. Appl. Physiol.* 79, 1497–1503. doi: 10.1152/jappl.1995.79.5.1497

Fehrenbach, E., Niess, A. M., Schlotz, E., Passek, F., Dickhuth, H.-H., and Northoff, H. (2000). Transcriptional and translational regulation of heat shock proteins in leukocytes of endurance runners. *J. Appl. Physiol.* 89, 704–710. doi: 10.1152/jappl.2000.89.2.704

Fernandes, M. S., de, S., Silva, L. de L. de S. e., Kubrusly, M. S., Lima, T. R. L., de, A., et al. (2020). Aerobic exercise training exerts beneficial effects upon oxidative metabolism and non-enzymatic antioxidant defense in the liver of leptin deficiency mice. *Front. Endocrinol. (Lausanne).* 11, 1–9. doi: 10.3389/fendo.2020.588502

Frydelund-Larsen, L., Penkowa, M., Akerstrom, T., Zankari, A., Nielsen, S., and Pedersen, B. K. (2007). Exercise induces interleukin-8 receptor (CXCR2) expression in human skeletal muscle. *Exp. Physiol.* 92, 233–240. doi: 10.1113/expphysiol.2006.034769

Gaggini, M., Vassalle, C., Carli, F., Maltinti, M., Sabatino, L., Buzzigoli, E., et al. (2021). Changes in Plasma Bioactive Lipids and Inflammatory Markers during a Half-Marathon in Trained Athletes. *Appl. Sci.* 11, 4622. doi: 10.3390/app11104622

Gill, S., Hankey, J., Wright, A., Marczak, S., Hemming, K., Allerton, D., et al. (2015a). The impact of a 24-h ultra-marathon on circulatory endotoxin and cytokine profile. *Int. J. Sports Med.* 36, 688–695. doi: 10.1055/s-0034-1398535

Gill, S. K., Teixeira, A., Rama, L., Prestes, J., Rosado, F., Hankey, J., et al. (2015b). Circulatory endotoxin concentration and cytokine profile in response to exertional-heat stress during a multi-stage ultra-marathon competition. *Exerc. Immunol. Rev.* 21, 114–28. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25830597.

Gleeson, M., Bishop, N. C., Stensel, D. J., Lindley, M. R., Mastana, S. S., and Nimmo, M. A. (2011). The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat. Rev. Immunol.* 11, 607–615. doi: 10.1038/nri3041

Glund, S., and Krook, A. (2007). Role of interleukin-6 signalling in glucose and lipid metabolism. *Acta Physiol.* 192, 37–48. doi: 10.1111/j.1748-1716.2007.01779.x

Gomarasca, M., Banfi, G., and Lombardi, G. (2020). "Myokines: The endocrine coupling of skeletal muscle and bone," in *Advances in Clinical Chemistry* (Elsevier *Inc.)* 155–218. doi: 10.1016/bs.acc.2019.07.010

Goussetis, E., Spiropoulos, A., Tsironi, M., Skenderi, K., Margeli, A., Graphakos, S., et al. (2009). Spartathlon, a 246 kilometer foot race: Effects of acute inflammation induced by prolonged exercise on circulating progenitor reparative cells. *Blood Cells, Mol. Dis.* 42, 294–299. doi: 10.1016/j.bcmd.2009.01.003

Henson, D. A., Nieman, D. C., Pistilli, E. E., Schilling, B., Colacino, A., Utter, A. C., et al. (2004). Influence of carbohydrate and age on lymphocyte function following a marathon. *Int. J. Sport Nutr. Exerc. Metab.* 14, 308–322. doi: 10.1123/ijsnem.14.3.308

Higgins, C. A., Judge, T. A., and Ferris, G. R. (2003). Influence tactics and work outcomes: a meta-analysis. *J. Organ. Behav.* 24, 89–106. doi: 10.1002/job.181

Higgins, J. P. T., Altman, D. G., Gotzsche, P. C., Juni, P., Moher, D., Oxman, A. D., et al. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 343, d5928–d5928. doi: 10.1136/bmj.d5928

Howatson, G., McHugh, M. P., Hill, J. A., Brouner, J., Jewell, A. P., Van Someren, K. A., et al. (2010). Influence of tart cherry juice on indices of recovery following marathon running. *Scand. J. Med. Sci. Sports* 20, 843–852. doi: 10.1111/j.1600-0838.2009.01005.x

Jamurtas, A. Z., Stavropoulos-Kalinoglou, A., Koutsias, S., Koutedakis, Y., and Fatouros, I. (2015). Adiponectin, resistin, and visfatin in childhood obesity and exercise. *Pediatr. Exerc. Sci.* 27, 454–462. doi: 10.1123/pes.2014-0072

Järvinen, T. A., Järvinen, M., and Kalimo, H. (2013). Regeneration of injured skeletal muscle after the injury. *Muscles. Ligaments Tendons J.* 3, 337–345. doi: 10.32098/mltj.04.2013.16

Kasprowicz, K., Ratkowski, W., Wołyniec, W., Kaczmarczyk, M., and Witek, K., Zmijewski, P., et al. (2020). The effect of vitamin D3 supplementation on hepcidin, iron, and IL-6 responses after a 100 km ultra-marathon. *Int. J. Environ. Res. Public Health* 17, 2962. doi: 10.3390/ijerph17082962

Kaufmann, C. C., Wegberger, C., Tscharre, M., Haller, P. M., Piackova, E., Vujasin, I., et al. (2021). Effect of marathon and ultra-marathon on inflammation and iron homeostasis. *Scand. J. Med. Sci. Sports* 31, 542–552. doi: 10.1111/sms.13869

Keller, C., Steensberg, A., Pilegaard, H., Osada, T., Saltin, B., Pedersen, B. K., et al. (2001). Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. *FASEB J.* 15, 1–15. doi: 10.1096/fj.01-0507fje

Kerschan-Schindl, K., Thalmann, M., Sodeck, G. H., Skenderi, K., Matalas, A. L., Grampp, S., et al. (2009). A 246-km continuous running race causes significant changes in bone metabolism. *Bone* 45, 1079–1083. doi: 10.1016/j.bone.2009.07.088

Kim, H. J., Lee, Y. H., and Kim, C. K. (2007). Biomarkers of muscle and cartilage damage and inflammation during a 200 km run. *Eur. J. Appl. Physiol.* 99, 443–447. doi: 10.1007/s00421-006-0362-y

Knechtle, B., and Nikolaidis, P. T. (2018). Physiology and pathophysiology in ultra-marathon running. *Front. Physiol.* 9, 634. doi: 10.3389/fphys.2018.00634

Larsen, E. L., Poulsen, H. E., Michaelsen, C., Kjær, L. K., Lyngbæk, M., Andersen, E. S., et al. (2020). Differential time responses in inflammatory and oxidative stress markers after a marathon: An observational study. *J. Sports Sci.* 38, 2080–2091. doi: 10.1080/02640414.2020.1770918

Leal, L. G., Lopes, M. A., and Batista, M. L. (2018). Physical exercise-induced myokines and muscle-adipose tissue crosstalk: a review of current knowledge and the implications for health and metabolic diseases. *Front. Physiol.* 9, 1–17. doi: 10.3389/fphys.2018.01307

Li, H.-Y., Hong, X., Cao, Q.-Q., and So, K.-F. (2019). "Adiponectin, exercise and eye diseases," in *International Review of Neurobiology* (Elsevier Inc.) p. 281–294. doi: 10.1016/bs.irn.2019.07.006

Luna Junior, L. A., de Melo Batista dos Santo, J., Lacerda Bachi, A. L., Foster, R., Slowetzky Amaro, A., Ligeiro de Oliveira, A. P., et al. (2016). Relationship between cytokines and running economy in marathon runners. *Open Life Sci.* 11, 308–312. doi: 10.1515/biol-2016-0041

Margeli, A., Skenderi, K., Tsironi, M., Hantzi, E., Matalas, A.-L., Vrettou, C., et al. (2005). Dramatic elevations of interleukin-6 and acute-phase reactants in athletes participating in the ultradistance foot race spartathlon: severe systemic inflammation and lipid and lipoprotein changes in protracted exercise. *J. Clin. Endocrinol. Metab.* 90, 3914–3918. doi: 10.1210/jc.2004-2346

Mastaloudis, A., Morrow, J. D., Hopkins, D. W., Devaraj, S., and Traber, M. G. (2004). Antioxidant supplementation prevents exercise-induced lipid peroxidation, but not inflammation, in ultramarathon runners. *Free Radic. Biol. Med.* 36, 1329–1341. doi: 10.1016/j.freeradbiomed.2004.02.069

Miles, M. P., Walker, E. E., Conant, S. B., Hogan, S. P., and Kidd, J. R. (2006). Carbohydrate influences plasma interleukin-6 but not C-reactive protein or creatine kinase following a 32-km mountain trail race. *Int. J. Sport Nutr. Exerc. Metab.* 16, 36–46. doi: 10.1123/ijsnem.16.1.36

Moldoveanu, A. I., Shephard, R. J., and Shek, P. N. (2001). The cytokine response to physical activity and training. *Sport. Med.* 31, 115–144. doi: 10.2165/00007256-200131020-00004

Neidhart, M., Müller-Ladner, U., Frey, W., Bosserhoff, A. K., Colombani, P. C., Frey-Rindova, P., et al. (2000). Increased serum levels of non-collagenous matrix proteins (cartilage oligomeric matrix protein and melanoma inhibitory activity) in marathon runners. *Osteoarthr. Cartil.* 8, 222–229. doi: 10.1053/joca.1999.0293

Ng, Q. Y., Lee, K. W., Byrne, C., Ho, T. F., and Lim, C. L. (2008). Plasma endotoxin and immune responses during a 21-km road race under a warm and humid environment. *Ann. Med. Singapore* 37, 307.

Nickel, T., Emslander, I., Sisic, Z., David, R., Schmaderer, C., Marx, N., et al. (2012). Modulation of dendritic cells and toll-like receptors by marathon running. *Eur. J. Appl. Physiol.* 112, 1699–1708. doi: 10.1007/s00421-011-2140-8

Nieman, D. C., Dumke, C. I., Henson, D. A., McAnulty, S. R., McAnulty, L. S., Lind, R. H., et al. (2003). Immune and Oxidative Changes During and Following the Western States Endurance Run. *Int. J. Sports Med.* 24, 541–547. doi: 10.1055/s-2003-42018

Nieman, D. C., Dumke, C. L., Henson, D. A., McAnulty, S. R., Gross, S. J., and Lind, R. H. (2005). Muscle damage is linked to cytokine changes following a 160-km race. *Brain. Behav. Immun.* 19, 398–403. doi: 10.1016/j.bbi.2005.03.008

Nieman, D. C., Henson, D. A., Davis, J. M., Dumke, C. L., Gross, S. J., Jenkins, D. P., et al. (2007). Quercetin Ingestion Does Not Alter Cytokine Changes in Athletes Competing in the Western States Endurance Run. *J. Interf. Cytokine Res.* 27, 1003–1012. doi: 10.1089/jir.2007.0050

Nieman, D. C., Henson, D. A., Dumke, C. L., Oley, K., McAnulty, S. R., Davis, J. M., et al. (2006). Ibuprofen use, endotoxemia, inflammation, and plasma cytokines during ultramarathon competition. *Brain. Behav. Immun.* 20, 578–584. doi: 10.1016/j.bbi.2006.02.001

Nieman, D. C., Henson, D. A., McAnulty, S. R., McAnulty, L., Swick, N. S., Utter, A. C., et al. (2002). Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. *J. Appl. Physiol.* 92, 1970–1977. doi: 10.1152/japplphysiol.00961.2001

Nieman, D. C., Henson, D. A., Smith, L. L., Utter, A. C., Vinci, D. M., Davis, J. M., et al. (2001). Cytokine changes after a marathon race. *J. Appl. Physiol.* 91, 109–114. doi: 10.1152/jappl.2001.91.1.109

Niemelä, M., Kangastupa, P., Niemel,ä, O., Bloigu, R., and Juvonen, T. (2016). Acute changes in inflammatory biomarker levels in recreational runners participating in a marathon or half-marathon. *Sport. Med. - Open* 2, 21. doi: 10.1186/s40798-016-0045-0

Niess, A. M., Passek, F., Lorenz, I., Schneider, E. M., Dickhuth, H.-H., Northoff, H., et al. (1999). Expression of the antioxidant stress protein heme oxygenase-1 (HO-1) in human leukocytes. *Free Radic. Biol. Med.* 26, 184–192. doi: 10.1016/S0891-5849(98)00192-0

Ostrowski, K., Rohde, T., Asp, S., Schjerling, P., and Pedersen, B. K. (1999). Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J. Physiol.* 515, 287–291. doi: 10.1111/j.1469-7793.1999.287ad.x

Ostrowski, K., Rohde, T., Zacho, M., Asp, S., and Pedersen, B. K. (1998). Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J. Physiol.* 508, 949–953. doi: 10.1111/j.1469-7793.1998.949bp.x

Ostrowski, K., Schjerling, P., and Pedersen, B. K. (2000). Physical activity and plasma interleukin-6 in humans - effect of intensity of exercise. *Eur. J. Appl. Physiol.* 83, 512–515. doi: 10.1007/s004210000312

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., et al. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* 372, n71. doi: 10.1136/bmj.n71

Papassotiriou, I., Alexiou, V. G., Tsironi, M., Skenderi, K., Spanos, A., and Falagas, M. E. (2008). Severe aseptic inflammation caused by long distance running (246 km) does not increase procalcitonin. *Eur. J. Clin. Invest.* 38, 276–279. doi: 10.1111/j.1365-2362.2008.01935.x

Passos, B. N., Lima, M. C., Sierra, A. P. R., Oliveira, R. A., Maciel, J. F. S., Manoel, R., et al. (2019). Association of Daily Dietary Intake and Inflammation Induced by Marathon Race. *Mediators Inflamm.* 2019, 1–8. doi: 10.1155/2019/1537274

Pedersen, B. K. (2011). Muscles and their myokines. *J. Exp. Biol.* 214, 337–346. doi: 10.1242/jeb.048074

Pedersen, B. K. (2016). "Myokines and Metabolism," in *Metabolic Syndrome* (Cham: Springer International Publishing) p. 541–554. doi: 10.1007/978-3-319-11251-0_31

Pedersen, B. K. (2017). Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. *Eur. J. Clin. Invest.* 47, 600–611. doi: 10.1111/eci.12781

Pedersen, B. K. (2019). Physical activity and muscle–brain crosstalk. *Nat. Rev. Endocrinol.* 15, 383–392. doi: 10.1038/s41574-019-0174-x

Pedersen, B. K., and Febbraio, M. A. (2008). Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol. Rev.* 88, 1379–1406. doi: 10.1152/physrev.90100.2007

Peters, E. M., Anderson, R., Nieman, D. C., Fickl, H., and Jogessar, V. (2001). Vitamin C supplementation attenuates the increases in circulating cortisol, adrenaline and anti-inflammatory polypeptides following ultramarathon running. *Int. J. Sports Med.* 22, 537–543. doi: 10.1055/s-2001-17610

Petersen, A. M. W., and Pedersen, B. K. (2005). The anti-inflammatory effect of exercise. *J. Appl. Physiol.* 98, 1154–1162. doi: 10.1152/japplphysiol.00164.2004

Pistilli, E. E., Nieman, D. C., Henson, D. A., Kaminsky, D. E., Utter, A. C., Vinci, D. M., et al. (2002). Influence of age on immune changes in runners after a marathon. *J. Aging Phys. Act.* 10, 432–442. doi: 10.1123/japa.10.4.432

Pngwing. (2021). https://www.pngwing.com/pt/free-png-blxjt/download (accessed December 09, 2021).

Pugh, J. N., Sparks, A. S., Doran, D. A., Fleming, S. C., Langan-Evans, C., Kirk, B., et al. (2019). Four weeks of probiotic supplementation reduces GI symptoms during a marathon race. *Eur. J. Appl. Physiol.* 119, 1491–1501. doi: 10.1007/s00421-019-04136-3

Reihmane, D., Jurka, A., Tretjakovs, P., and Dela, F. (2013). Increase in IL-6, TNF-α, and MMP-9, but not sICAM-1, concentrations depends on exercise duration. *Eur. J. Appl. Physiol.* 113, 851–858. doi: 10.1007/s00421-012-2491-9

Roupas, N., Mamali, I., Maragkos, S., Leonidou, L., Armeni, A., Markantes, G., et al. (2013). The effect of prolonged aerobic exercise on serum adipokine levels during an ultra-marathon endurance race. *Hormones* 12, 275–282. doi: 10.14310/horm.2002.1411

Sansoni, V., Vernillo, G., Perego, S., Barbuti, A., Merati, G., Schena, F., et al. (2017). Bone turnover response is linked to both acute and established metabolic changes in ultra-marathon runners. *Endocrine* 56, 196–204. doi: 10.1007/s12020-016-1012-8

Santos, V. C., Levada-Pires, A. C., Alves, S. R., Pithon-Curi, T. C., Curi, R., and Cury-Boaventura, M. F. (2013a). Changes in lymphocyte and neutrophil function induced by a marathon race. *Cell Biochem. Funct.* 31, 237–243. doi: 10.1002/cbf.2877

Santos, V. C., Levada-Pires, A. C., Alves, S. R., Pithon-Curi, T. C., Curi, R., and Cury-Boaventura, M. F. (2013b). Effects of DHA-rich fish oil supplementation on lymphocyte function before and after a marathon race. *Int. J. Sport Nutr. Exerc. Metab.* 23, 161–169. doi: 10.1123/ijsnem.23.2.161

Santos, V. C., Sierra, A. P. R., Oliveira, R., Caçula, K. G., Momesso, C. M., Sato, F. T., et al. (2016). Marathon race affects neutrophil surface molecules: role of inflammatory mediators. *PLoS ONE.* 11, e0166687. doi: 10.1371/journal.pone.0166687

Saraiva, M., and O'Garra, A. (2010). The regulation of IL-10 production by immune cells. *Nat. Rev. Immunol.* 10, 170–181. doi: 10.1038/nri2711

Scherr, J., Braun, S., Schuster, T., Hartmann, C., Moehlenkamp, S., Wolfarth, B., et al. (2011). 72-h Kinetics of high-sensitive troponin T and inflammatory markers after marathon. *Med. Sci. Sport. Exerc.* 43, 1819–1827. doi: 10.1249/MSS.0b013e31821b12eb

Scherr, J., Nieman, D. C., Schuster, T., Habermann, J., Rank, M., Braun, S., et al. (2012). Nonalcoholic beer reduces inflammation and incidence of respiratory tract illness. *Med. Sci. Sports Exerc.* 44, 18–26. doi: 10.1249/MSS.0b013e3182250dda

Shanely, R. A., Nieman, D. C., Zwetsloot, K. A., Knab, A. M., Imagita, H., Luo, B., et al. (2014). Evaluation of Rhodiola rosea supplementation on skeletal muscle damage and inflammation in runners following a competitive marathon. *Brain. Behav. Immun.* 39, 204–210. doi: 10.1016/j.bbi.2013.09.005

Shin, Y. O., and Lee, J. B. (2013). Leukocyte chemotactic cytokine and leukocyte subset responses during ultra-marathon running. *Cytokine.* 61, 364–369. doi: 10.1016/j.cyto.2012.11.019

Shoghi, K. I., Gropler, R. J., Sharp, T., Herrero, P., Fettig, N., Su, Y., et al. (2008). Time course of alterations in myocardial glucose utilization in the zucker diabetic fatty rat with correlation to gene expression of glucose transporters: a small-animal PET investigation. *J. Nucl. Med.* 49, 1320–1327. doi: 10.2967/jnumed.108.051672

Siegel, A. J., Verbalis, J. G., Clement, S., Mendelson, J. H., Mello, N. K., Adner, M., et al. (2007). Hyponatremia in marathon runners due to inappropriate arginine vasopressin secretion. *Am. J. Med. 120* 461.e11-461.e17. doi: 10.1016/j.amjmed.2006.10.027

Sierra, A. P., Oliveira-Junior, M. C., Almeida, F. M., Benetti, M., Oliveira, R., Felix, S. N., et al. (2019a). Impairment on cardiopulmonary function after marathon: role of exhaled nitric oxide. *Oxid. Med. Cell. Longev.* 2019. doi: 10.1155/2019/5134360

Sierra, A. P. R., Lima, G. H. O., da Silva, E. D., Maciel, J. F., de, S., Benetti, M. P., et al. (2019b). Angiotensin-converting enzyme related-polymorphisms on inflammation, muscle and myocardial damage after a marathon race. *Front. Genet.* 10, 1–12. doi: 10.3389/fgene.2019.00984

Skinner, S., Nader, E., Stauffer, E., Robert, M., Boisson, C., Cibiel, A., et al. (2021). Differential impacts of trail and ultra-trail running on cytokine profiles: An observational study. *Clin. Hemorheol. Microcirc.* 78, 301–310. doi: 10.3233/CH-211121

Sliwicka, E., Ciso,ń, T., Pilaczyńska-Szcześniak, Ł., Ziemba, A., and Straburzyńska-Lupa, A. (2021). Effects of marathon race on selected myokines and sclerostin in middle-aged male amateur runners. *Sci. Rep.* 11, 1–9. doi: 10.1038/s41598-021-82288-z

Smith, K. A., Kisiolek, J. N., Willingham, B. D., Morrissey, M. C., Leyh, S. M., Saracino, P. G., et al. (2020). Ultra-endurance triathlon performance and markers of whole-body and gut-specific inflammation. *Eur. J. Appl. Physiol.* 120, 349–357. doi: 10.1007/s00421-019-04279-3

Smith, L. L. (2000). Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? *Med. Sci. Sport. Exerc.* 32, 317. doi: 10.1097/00005768-200002000-00011

Starkie, R. L., Rolland, J., Angus, D. J., Anderson, M. J., and Febbraio, M. A. (2001). Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-α levels after prolonged running. *Am. J. Physiol. Physiol.* 280, C769–C774. doi: 10.1152/ajpcell.2001.280.4.C769

Steensberg, A., Febbraio, M. A., Osada, T., Schjerling, P., Hall, G., Saltin, B., et al. (2001). Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. *J. Physiol.* 537, 633–639. doi: 10.1111/j.1469-7793.2001.00633.x

Suzuki, K., Nakaji, S., Yamada, M., Liu, Q., Kurakake, S., Okamura, N., et al. (2003). Impact of a competitive marathon race on systemic cytokine and neutrophil responses. *Med. Sci. Sports Exerc.* 35, 348–355. doi: 10.1249/01.MSS.0000048861.57899.04

Suzuki, K., Yamada, M., Kurakake, S., Okamura, N., Yamaya, K., Liu, Q., et al. (2000). Circulating cytokines and hormones with immunosuppressive but neutrophil-priming potentials rise after endurance exercise in humans. *Eur. J. Appl. Physiol.* 81, 281–287. doi: 10.1007/s004210050044

Tanner, E. A., Gary, M. A., Davis, A. A., Michalik, S., and McFarlin, B. K. (2021). Alterations in systemic inflammatory response following a half-marathon race with a combined curcumin and pomegranate supplement: a feasibility study. *J. Diet. Suppl.* 18, 461–477. doi: 10.1080/19390211.2020.1786206

Tavares-Silva, E., Caris, A. V., Santos, S. A., Ravacci, G. R., and Thomatieli-Santos, R. V. (2021). Effect of multi-strain probiotic supplementation on URTI symptoms and cytokine production by monocytes after a marathon race: a randomized, double-blind, placebo study. *Nutrients* 13, 1478. doi: 10.3390/nu13051478

Terra, R., Silva, S. A. G., da, Pinto, V. S., and Dutra, P. M. L. (2012). Efeito do exercício no sistema imune: resposta, adaptação e sinalização celular. *Rev. Bras. Med. do Esporte* 18, 208–214. doi: 10.1590/S1517-86922012000300015

Toft, A. D., Thorn, M., Ostrowski, K., Asp, S., Møller, K., Iversen, S., et al. (2000). N-3 polyunsaturated fatty acids do not affect cytokine response to strenuous exercise. *J. Appl. Physiol.* 89, 2401–2406. doi: 10.1152/jappl.2000.89.6.2401

Turcotte, L. P., and Fisher, J. S. (2008). Skeletal muscle insulin resistance: roles of fatty acid metabolism and exercise. *Phys. Ther.* 88, 1279–1296. doi: 10.2522/ptj.20080018

Uchakin, P. N., Gotovtseva, E. P., and Stray-Gundersen, J. (2003). Immune and neuroendocrine alterations in marathon runners. *J. Appl. Res.* 3, 483–494.

Uchida, M., Oyanagi, E., Kawanishi, N., Iemitsu, M., Miyachi, M., Kremenik, M. J., et al. (2014). Exhaustive exercise increases the TNF-α production in response to flagellin via the upregulation of toll-like receptor 5 in the large intestine in mice. *Immunol. Lett.* 158, 151–158. doi: 10.1016/j.imlet.2013.12.021

Vaisberg, M., Bachi, A. L. L., Latrilha, C., Dioguardi, G. S., Bydlowski, S. P., and Maranhão, R. C. (2012). Lipid transfer to HDL is higher in marathon runners than in sedentary subjects, but is acutely inhibited during the run. *Lipids* 47, 679–686. doi: 10.1007/s11745-012-3685-y

Vaisberg, M., Suguri, V. M., Gregorio, L. C., Lopes, J. D., and Bachi, A. L. L. (2013). Cytokine kinetics in nasal mucosa and sera: new insights in understanding upper-airway disease of marathon runners. *Exerc. Immunol. Rev.* 19, 49–59. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23977719.

Vuolteenaho, K., Leppänen, T., Kekkonen, R., Korpela, R., and Moilanen, E. (2014). Running a marathon induces changes in adipokine levels and in markers of cartilage degradation – novel role for resistin. *PLoS ONE.* 9, e110481. doi: 10.1371/journal.pone.0110481

Weigert, C., Hennige, A. M., Brodbeck, K., Häring, H. U., and Schleicher, E. D. (2005). Interleukin-6 acts as insulin sensitizer on glycogen synthesis in human skeletal muscle cells by phosphorylation of Ser 473 of Akt. *Am. J. Physiol. Metab.* 289, E251–E257. doi: 10.1152/ajpendo.00448.2004

Weinstock, C., KöNig, D., Harnischmacher, R., Keul, J., Berg, A., and Northoff, H. (1997). Effect of exhaustive exercise stress on the cytokine response. *Med. &amp Sci. Sport. &amp Exerc.* 29, 345–354. doi: 10.1097/00005768-199703000-00009

Wilhelm, M., Zueger, T., De Marchi, S., Rimoldi, S. F., Brugger, N., Steiner, R., et al. (2014). Inflammation and atrial remodeling after a mountain marathon. *Scand. J. Med. Sci. Sports* 24, 519–525. doi: 10.1111/sms.12030

Wołyniec, W., Ratkowski, W., Kasprowicz, K., Małgorzewicz, S., Aleksandrowicz, E., Zdrojewski, T., et al. (2020). Factors influencing post-exercise proteinuria after marathon and ultramarathon races. *Biol. Sport* 37, 33–40. doi: 10.5114/biolsport.2020.89939

Wu, R., Li, N., Zhao, X., Ding, T., Xue, H., Gao, C., et al. (2020). Low-dose Interleukin-2: Biology and therapeutic prospects in rheumatoid arthritis. *Autoimmun. Rev.* 19, 102645. doi: 10.1016/j.autrev.2020.102645

Yargic, M. P., Torgutalp, S., Akin, S., Babayeva, N., Torgutalp, M., and Demirel, H. A. (2019). Acute long-distance trail running increases serum IL-6, IL-15, and Hsp72 levels. *Appl. Physiol. Nutr. Metab.* 44, 627–631. doi: 10.1139/apnm-2018-0520

Yetgin, M. K., Agopyan, A., Kucukler, F. K., Gedikbasi, A., Yetgin, S., Kayapinar, F. C., et al. (2018). The influence of physical training modalities on basal metabolic rate and leptin on obese adolescent boys. *J. Pak. Med. Assoc.* 68, 929–931.

Zaccaria, M., Ermolao, A., Roi, G. S., Englaro, P., Tegon, G., and Varnier, M. (2002). Leptin reduction after endurance races differing in duration and energy expenditure. *Eur. J. Appl. Physiol.* 87, 108–111. doi: 10.1007/s00421-002-0606-4

Zaldivar, F., Wang-Rodriguez, J., Nemet, D., Schwindt, C., Galassetti, P., Mills, P. J., et al. (2006). Constitutive pro- and anti-inflammatory cytokine and growth factor response to exercise in leukocytes. *J. Appl. Physiol.* 100, 1124–1133. doi: 10.1152/japplphysiol.00562.2005

Zhang, Y., and Chua, S. (2017). "Leptin Function and Regulation," in *Comprehensive Physiology (Wiley)* 351–369. doi: 10.1002/cphy.c160041

Zimmer, P., Baumann, F. T., Bloch, W., Zopf, E. M., Schulz, S., Latsch, J., et al. (2016). Impact of a half marathon on cellular immune system, pro-inflammatory cytokine levels, and recovery behavior of breast cancer patients in the aftercare compared to healthy controls. *Eur. J. Haematol.* 96, 152–159. doi: 10.1111/ejh.12561

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HYPOTHESIS AND THEORY
published: 10 March 2022
doi: 10.3389/fphys.2022.843784

Potential Role of Chronic Physical Exercise as a Treatment in the Development of Vitiligo

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Vitiligo is an autoimmune disease characterized by progressive skin depigmentation and the appearance of white patches throughout the body caused by significant apoptosis of epidermal melanocytes. Despite not causing any physical pain, vitiligo can originate several psychosocial disorders, drastically reducing patients' quality of life. Emerging evidence has shown that vitiligo is associated with several genetic polymorphisms related to auto-reactivity from the immune system to melanocytes. Melanocytes from vitiligo patients suffer from excess reactive oxygen species (ROS) produced by defective mitochondria besides a poor endogenous antioxidant system (EAS). This redox imbalance results in dramatic melanocyte oxidative stress (OS), causing significant damage in proteins, lipid membranes, and DNA. The damaged melanocytes secret damage-associated molecular pattern (DAMPs), inducing and increasing inflammatory gene expression response that ultimately leads to melanocytes apoptosis. Vitiligo severity has been also associated with increasing the prevalence and incidence of metabolic syndrome (MetS) or associated disorders such as insulin resistance and hypercholesterolemia. Thus, suggesting that in genetically predisposed individuals, the environmental context that triggers MetS (i.e., sedentary lifestyle) may also be an important trigger for the development and severity of vitiligo disease. This paper will discuss the relationship between the immune system and epidermal melanocytes and their interplay with the redox system. Based on state-of-the-art evidence from the vitiligo research, physical exercise (PE) immunology, and redox system literature, we will also propose chronic PE as a potential therapeutic strategy to treat and prevent vitiligo disease progression. We will present evidence that chronic PE can change the balance of inflammatory to an anti-inflammatory state, improve both EAS and the mitochondrial structure and function (resulting in the decrease of OS). Finally, we will highlight clinically relevant markers that can be analyzed in a new research avenue to test the potential applicability of chronic PE in vitiligo disease.

Keywords: vitiligo, autoimmune disease, physical training, immune system, oxidative stress, metabolic syndrome

OPEN ACCESS

Edited by:
Ana Cláudia Garcia De Oliveira Duarte, Federal University of São Carlos, Brazil

Reviewed by:
Anand Thirupathi, Ningbo University, China
Kanhaiya Singh, Indiana University, Purdue University Indianapolis, United States

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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *26 December 2021*
Accepted: *08 February 2022*
Published: *10 March 2022*

Citation:
de França E, dos Santos RVT, Baptista LC, Da Silva MAR, Fukushima AR, Hirota VB, Martins RA and Caperuto EC (2022) Potential Role of Chronic Physical Exercise as a Treatment in the Development of Vitiligo. Front. Physiol. 13:843784. doi: 10.3389/fphys.2022.843784

INTRODUCTION

Vitiligo is an autoimmune disease characterized by progressive skin depigmentation and the appearance of white patches throughout the body, caused by significant apoptosis of epidermal melanocytes (Bergqvist and Ezzedine, 2020). Currently, it is estimated that vitiligo affects 0.5–2% of the global population, being the most prevalent skin disease (Krüger and Schallreuter, 2012), and despite not causing any physical pain, vitiligo can generate several psychosocial disorders reducing drastically patients' quality of life (Krüger and Schallreuter, 2013).

There is no known cure for vitiligo, but there are several management strategies to reduce the spread of white skin patches and to attempt to re-pigmentate the affected areas. The most used treatments are immunosuppressive drugs such as corticosteroids, calcineurin inhibitors, antioxidant supplements, and phototherapies. However, these therapeutics are not 100% effective and do not prevent the disease reappearance (Bergqvist and Ezzedine, 2020). Therefore, it is still needed cost-effective strategies to prevent the resurgence of vitiligo wounds and effectively stop the spread of white patches. Knowing the underlying molecular mechanisms and patients 'environmental context is essential to develop effective treatments.

Emerging evidence has shown that vitiligo is associated with several genetic polymorphisms related to auto-reactivity from the immune system (IS) to melanocytes. Melanocytes from vitiligo patients suffer from excess reactive oxygen species (ROS) produced by defective mitochondria besides a poor endogenous antioxidant system (EAS) (Bergqvist and Ezzedine, 2020). This redox imbalance results in dramatic melanocyte oxidative stress (OS), causing significant damage in proteins, lipid membranes, and DNA. The damaged melanocytes secret damage-associated molecular pattern (DAMPs), inducing and increasing inflammatory gene expression response that ultimately leads to cell apoptosis (Bergqvist and Ezzedine, 2020).

Vitiligo severity has also been associated with metabolic syndrome (MetS), increasing the prevalence and incidence of MetS or associated disorders such as insulin resistance and hypercholesterolemia (Ataş and Gönül, 2017; Sharma et al., 2017; Tanacan and Atakan, 2020; Verma et al., 2021). Etiologically, MetS are associated with a sedentary lifestyle (Edwardson et al., 2012), cellular inflammation, and OS mechanisms (Bonomini et al., 2015) that may be involved in the onset of vitiligo. Therefore, we speculate that in genetically predisposed individuals, the environmental context that triggers MetS (i.e., sedentary lifestyle) may also be an important trigger for the development and severity of vitiligo disease.

In this paper, we will discuss the molecular mechanisms and the role of patients' environmental context for the onset of vitiligo. Specifically, we will discuss the relationship between the immune system and epidermal melanocytes and their interplay with the redox system. Based on state-of-the-art evidence from the vitiligo research, physical exercise (PE) immunology, and redox system literature, we also propose *PE* as a potential therapeutic strategy to fight vitiligo adverse events (e.g., spreading new white skin wounds and associated comorbidities). We hypothesize that three potential changes induced by chronic PE can occur in vitiligo patients: (i) a positive immunomodulatory response (change the balance of inflammatory to an anti-inflammatory state), (ii) improvement in EAS, and (iii) improvement in the mitochondrial structure and function (resulting in the decrease of OS). Clinically, these biochemistry/metabolic/structural changes promoted by chronic PE training can stabilize the vitiligo, prevent the spread or reappearance of vitiligo after its stabilization and potentially promote the repigmentation of affected areas. Therefore, this paper highlights the clinical applicability of a structured PE training in vitiligo disease development and proposes a new research avenue to explore the potential role of PE training in vitiligo pathophysiological mechanisms and as treatment strategy.

OVERVIEW OF VITILIGO DEVELOPMENT

Several genetic polymorphisms have been identified in vitiligo development; such alterations come from the innate and adaptive IS and melanocytes morphology and metabolism (Bergqvist and Ezzedine, 2020).

In 2007, alterations in the NALP1 genomic region were identified in vitiligo patients (Jin et al., 2007). NALP1 encodes the NACHT Leucine-rich repeat protein 1 (a cytosolic pattern recognition receptors, which are highly expressed in T cells and Langerhans cells) that detect infection or cell damage in the cytosol (e.g., DAMPs). NACHT Leucine-rich repeat protein 1 recognize pathogen-associated molecular patterns and DAMPs and recruit other proteins to form signaling complexes that promote inflammation or type I interferon production (Jin et al., 2007; Abbas et al., 2019b). Posteriorly, an upregulation in interferon-gamma (IFN-γ) gene expression has been identified in the serum from vitiligo patients (Dwivedi et al., 2013). Further, low expression of CTLA-4 (cytotoxic T lymphocyte antigen-4) in T cells was also associated with higher vitiligo disease susceptibility (Ni et al., 2014). It is well established that NACHT actively promotes IL-1β and IFN-γ gene expression. IFN-γ, in turn, induces B7 gene expression in antigen-presenting cells (APC) in the epidermis (also known as Langerhans' cells) (Deng et al., 2018). Thus, low CTLA-4 expression in cytotoxic T cells (Song et al., 2013) increases the incidence of APC antigen presentation and cytotoxic T cells activation via B7 (from APC) to CD28 cytotoxic T cell binding (Abbas et al., 2019a, see **Figure 1**). Consequently, when this immunometabolism occurs with antigen from melanocytes, immune self-tolerance is lost, and melanocytes apoptosis occurs via accessory pathway activation. In contrast, in a vitiligo mouse model, it was demonstrated that an increase in regulatory T cells (Tregs) suppresses autoreactive cytotoxic T cells responses (Le Poole and Mehrotra, 2017). However, compared to healthy peers, vitiligo patients have low Tregs gene expression (and CTLA-4, as previously mentioned), which has a significant role in vitiligo development (Giri et al., 2020). Future studies are needed to explore a better strategy to promote increases in Tregs from vitiligo patients as illustrated in **Figure 1B**.

In vitiligo patients, high IFN-γ expression has been implicated in an aggressive and permanent IS response (IFN-γ → CXCL10

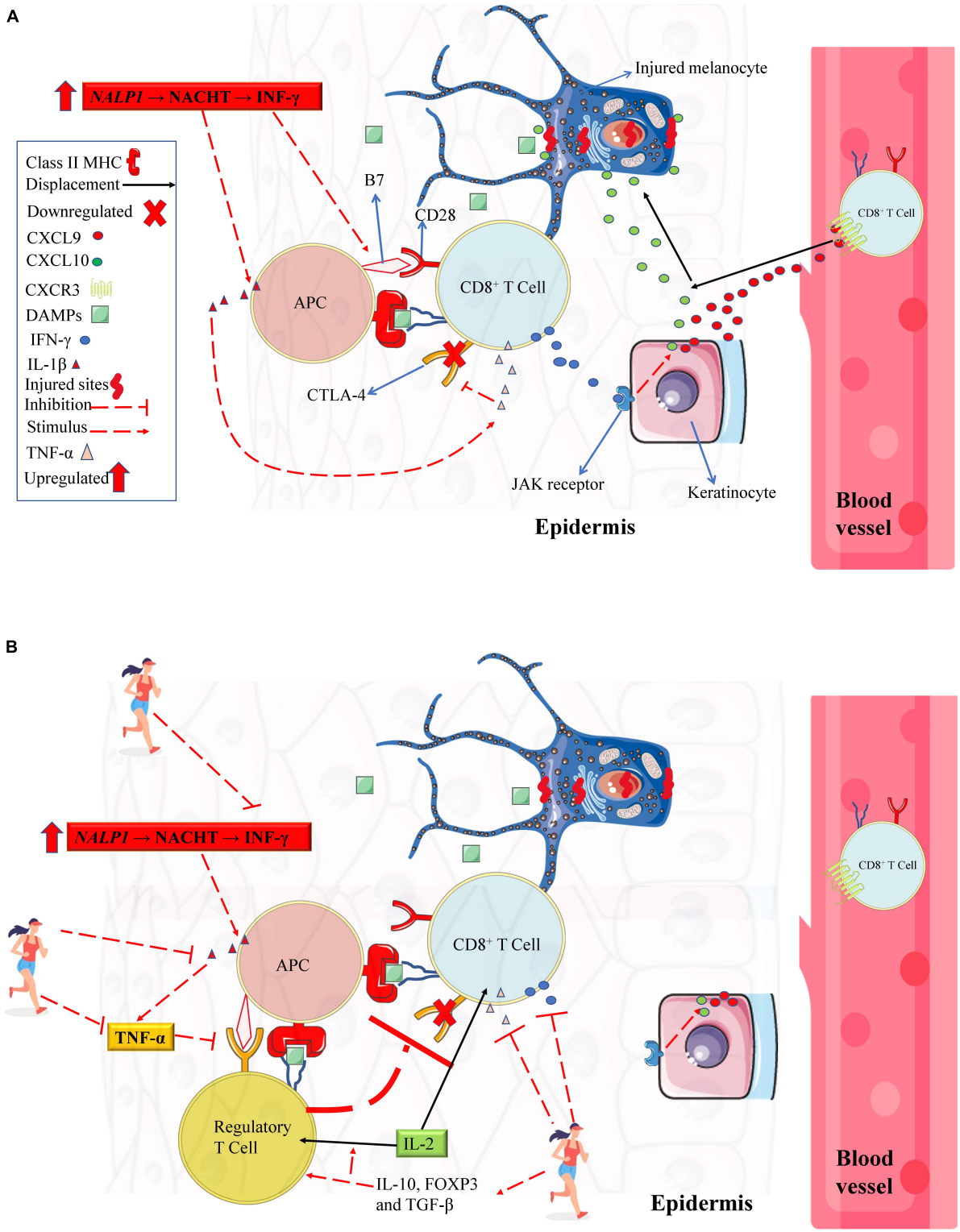

FIGURE 1 | Immune system profile in melanocytes from vitiligo patients. **(A)** Vitiligo patients have genetic polymorphisms that downregulate CTLA-4 from $CD8^+$ T cell and a mutation of the NALP1 gene (which results in a higher IFN-γ gene expression). These alterations might result in a greater IFN- γ gene expression upregulating the costimulatory molecule B7 from APC. Upon antigen presentation, the APC activates $CD8^+$ T cell via B7-CD28 interaction (in contrast, if B7 binds to CTLA-4 receptor, the $CD8^+$ T cell remains inactivated albeit antigen presentation). Upon activation, $CD8^+$ T cell secrets large amounts of IFN-γ, stimulating keratinocytes (via JAK/STAT pathway) to secret chemokines (CXCL9 and CXCL10) that recruit recirculation effector $CD8^+$ T cell in injured melanocytes inducing its

(Continued)

FIGURE 1 | apoptosis. In addition, activated $CD8^+$ T cells secrets large amounts of TNF-α, resulting in further downregulation in CTLA-4 receptors. **(B)** A theoretical model describing the potential role of chronic physical exercise on cytotoxic T cells suppressing. Chronic physical exercise (PE) decreases pro-inflammatory cytokines such as IL-β, IFN-γ, and TNF-α and increases anti-inflammatory IL-10. Inhibiting IL-1-β and TNF-α, there are no negative feedback for CTL-4 expression in cytotoxic T cell or regulatory T cell. When the CTL-4 receptor interacts with costimulatory molecule B7 from APC (because costimulatory molecule B7 has a greater affinity for CTLA-4 than the CD28 receptor), the regulatory T cell suppresses $CD8^+$ T cell activation. Also, due to chronic PE, the increase in IL-10, FOXP3, and TGF-β can stimulate regulatory T cells' transcription, differentiation, and proliferation. An increase in regulatory T cells suppresses autoreactive cytotoxic T cells responses. The increase in regulatory T cells can consume IL-2, which is important for maintaining memory T cells, thus potentiating the decrease in the excessive memory T cell as discussed in the paper. APC, antigen-presenting cell; CD28, Cluster of Differentiation 28; CTLA-4, cytotoxic T lymphocyte antigen-4; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor; DAMPs, damage-associated molecular pattern; JAK/STAT, Janus kinase/signal transducer and activator of transcription; IFN-γ, interferon-gamma; IL, interleukin; MHC, major histocompatibility complex; TGF-β, transforming growth factor, TNF-α, tumor necrosis factor-alpha. Figure created with images from smart.servier.com and storyset.com.

(C-X-C Motif Chemokine Ligand 10) → $CD8^+$ T cells) (Xie et al., 2016) targeting the population of epithelial cells throughout the body causing visual (Agarwala and Malkud, 2020), hearing (Ma et al., 2021) and vascular dysfunction (Azzazi et al., 2021). Nonetheless, one of the main visible features of this clinical condition is noted when the IS attacks epidermal melanocytes (Dwivedi et al., 2013), leading them to apoptosis without replacement, a phenotypic trait of vitiligo, identified by white spots in the skin, which signalize the absence of melanocytes at the site (Rashighi et al., 2014). The mechanistic trigger for loss of immune self-tolerance (which induces melanocytes apoptosis) is dysfunctional mitochondria resident in epidermal melanocytes (Dell'Anna et al., 2007) and also in $CD8^+$ T cells (Dell'Anna et al., 2003). These defective mitochondria have low concentration and abnormal cardiolipin distribution in the mitochondrial electron transport chain (mETC), which cause defects in complex I formation, impairing the stability and creation of mitochondria supercomplexes (essential for normal ATP production and low mitochondrial ROS emission (see scheme in **Figure 2**; Dell'Anna et al., 2003, 2007; Dell'Anna et al., 2017). Interestingly, in an *in vitro* study, cardiolipin replacement rescued normal mitochondria function from vitiligo patients (Dell'Anna et al., 2017). However, more studies are needed to determine the cause of low cardiolipin concentration in mitochondria from melanocytes and $CD8^+$ T cells and how to restore the normal concentrations of this lipid *in vivo*.

In addition to high mitochondrial ROS emission, vitiligo patients have a deficient EAS, with low activity and low gene expression of catalase (CAT), glutathione peroxidase (GPx), and thioredoxin reductase (TrxR) (Laddha et al., 2013; Xie et al., 2016), leading to a chronic OS state (Schallreuter, 2014). The low EAS effectiveness in vitiligo patients is related to its disabling caused by the chronic OS (Schallreuter et al., 1991a; Schallreuter, 2014). High OS causes cell damage leading melanocytes to secrete autoantigens such as calreticulin and heat shock protein 70 (Hsp70) acting as DAMPs (Mosenson, 2013; Zhang et al., 2014; Xie et al., 2016), which induces an adaptive immune response, via cytokines such as interleukin (IL)-1β, IL-6, IL-12, interferon (IFN)-α, IFN-γ, and tumor necrosis factor-alpha (TNF-α) (Asea, 2007; Xie et al., 2016).

A Possible Link Between Vitiligo Onset and Progression With Sedentary Lifestyle

As with other diseases, patients' environmental context modulates their genetic predisposition. Despite the consensus on the role of genetic predisposition and the greater susceptibility of vitiligo development, in monozygotic twins, vitiligo develops in only 23% of both twins (Alkhateeb et al., 2003), suggesting that the environment can induce or suppress the genes related to vitiligo development via DNA methylation, histone modifications and alteration of circulating microRNA (Zhou et al., 2019). For example, it has been identified that there is global hypermethylation of DNA in PBMCs, particularly in regions related to the increase of IL-10 in vitiligo patients (Zhao et al., 2010). Also, when compared to their healthy peers, vitiligo patients have a high serological concentration (Shi et al., 2016) and different miRNA profile expression in PBMC (Shi et al., 2013) that can characterize this population. The vitiligo miRNAs profile is related to melanocyte metabolism (Shi et al., 2016) and immune system regulation (Wang et al., 2015), such as cytokine profile to $CD8^+$ T cell upregulation in PBMCs (Zhou et al., 2019; Zhang et al., 2021) and melanocytes degeneration (Wang et al., 2015). It is well known that chronic PE also imposes strong epigenetic alteration on the immune system (Antrobus et al., 2021), EAS enzymes (Dimauro et al., 2020), and mitochondrial structure and function (Pareja-Galeano et al., 2014) throughout DNA methylation, post-translational histone modification, and microRNA transcripts. For example, data from the literature demonstrate that acute exercise induces PBMCs hypomethylation (Horsburgh et al., 2015), which might upregulate IL-10 expression in vitiligo patients (Zhao et al., 2010). In fact, vitiligo development has an epigenetic background that leads to its development, and it is plausible that chronic PE can recover it. Therefore, future studies deepening discussing this topic (if chronic PE is capable of rescuing the healthy epigenetic profile of vitiligo patients) is guaranteed and highly needed.

Today, little attention has been paid to the role of environmental factors like diet or PE training or the level of habitual physical activity in vitiligo disease. In fact, to the best of our knowledge, there are no studies focused on PE or habitual physical activity level and vitiligo disease, although several studies with similar etiological factors have consistently identified significant associations between vitiligo (as well as its severity) with MetS (Ataş and Gönül, 2017; Sharma et al., 2017; Tanacan and Atakan, 2020; Verma et al., 2021) or related dysfunctions such as higher blood plasma concentrations of low-density lipoprotein (LDL- cholesterol), low high-density lipoprotein (HDL-cholesterol), and insulin resistance (Karadag et al., 2011; Azzazi et al., 2021; Demirbaş et al., 2021; D'Arino et al., 2021). It is well established that a mainly sedentary lifestyle behavior

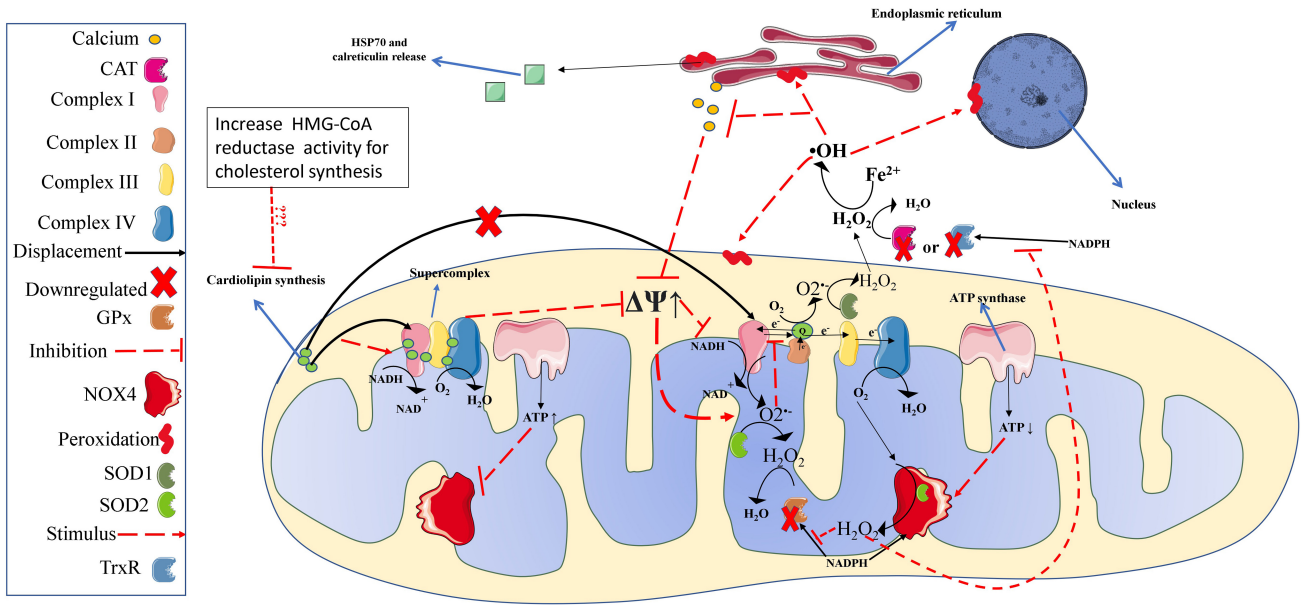

FIGURE 2 | Redox balance in melanocytes from vitiligo patients. Mitochondria of melanocytes from vitiligo patients have significant defects in complex I, which generate high amounts of ROS. Lower production and distribution of cardiolipin, which plays an essential role in complex I (and supercomplexes) structure and function, are responsible for the high mitochondrial superoxide production. However, superoxide production is efficiently amortized by SOD activity, converting them into hydrogen peroxide. In Vitiligo patients, NOX4 produces significant amounts of hydrogen peroxide (from oxygen); however, the higher NOX4 activity consumes NADPH molecules used as CAT, GPx, and TrxR cofactors in the hydrogen peroxide buffer activity. Also, hydrogen peroxide excess impairs CAT, GPx, and TrxR buffer activity. Thus, the hydrogen peroxide can reach critical values undergoing significant Fento reactions producing large amounts of hydroxyl radical, leading proteins, membranes (mitochondrial and cellular), and DNA peroxidation (oxidative stress). In melanocytes from vitiligo patients, the oxidative stress leads to a loss of calcium metabolism, which will imply a worsening in the mitochondrial structuring and function (i.e., further increase the ROS production associated with lower ATP levels). Such conditions lead melanocyte organelles to significant damage and release calreticulin and HSP70, acting as DAMPs and activating the immune response described in **Figure 1** (references are provided throughout the text). OH, hydroxyl radical; mitochondrial membrane potential; ATP, adenosine triphosphate; CAT, catalase; DAMPs, damage-associated molecular patterns; GPx, glutathione peroxidase, Fe^{2+}, iron ferrous; H_2O, water; H_2O_2, hydrogen peroxide; HSP70, Heat shock protein 70; NAD^+, oxidized form of nicotinamide adenine dinucleotide; NADH, reduced form of nicotinamide adenine dinucleotide; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; NOX2, NADPH oxidase isoform 2; NOX4, NADPH oxidase isoform 4; $O2^-$, superoxide, PE, physical exercise; ROS, reactive oxygen species; SOD, superoxide dismutase; TrxR, thioredoxin reductase.

and poor nutritional patterns are the most important risk factors for the onset of MetS or cholesterol disorders (Lira et al., 2010; Edwardson et al., 2012). Further, OS and the predominance of pro-inflammatory cytokines are also established mechanistic factors involved in MetS development (Bonomini et al., 2015). There is no study identifying a cause/effect relationship between MetS and vitiligo although vitiligo severity is associated with MetS. However, the incidence and prevalence of MetS or associated risk factors such as insulin resistance, hypertension, LDL-cholesterol, obesity, or large abdominal circumference are higher in vitiligo patients (Ataş and Gönül, 2017; Sharma et al., 2017; Tanacan and Atakan, 2020; Namazi et al., 2021). It is plausible that excessive ROS from other metabolic disorders (such as obesity, insulin resistance, and elevated LDL-cholesterol and low HDL-cholesterol) can induce a state of chronic low-grade inflammation (e.g., raising IFN-γ and TNF-α), possibly aggravating or activating vitiligo in genetically predisposed individuals, as illustrated in **Figure 1**. As discussed, and partially supporting this rationale, high levels of OS and IFN-γ are the mechanistic trigger for vitiligo manifestation (Laddha et al., 2014; Xie et al., 2016). Also, data from vitiligo patients in Dell'Anna et al. (2007, 2010), *in vitro* mechanistic study (Hauff et al., 2009), and animal model (Han et al., 2007) indicated that higher demand for cholesterol synthesis (e.g., for cell grow or LDL-cholesterol increases) is detrimental to mitochondrial cardiolipin content. This rationale is supported by data who statin mitigating the vitiligo spreading (Hasan et al., 2021). Statins have a lower effect on LDL-cholesterol via HMG-CoA reductase inhibition. HMG-CoA reductase is the rate limit for cholesterol synthesis and competes with cardiolipin for the cardiolipin synthase function (Hauff et al., 2009). Therefore, a link between lifestyle and vitiligo exists, and future experimental studies are needed to verify these hypotheses.

In vitiligo patients, these three factors (hyper-reactive IS, chronic OS, and deficient EAS) interact in an interdependent and reciprocal way. For instance, the increase in OS through an augment in defective mitochondria and an inefficient EAS trigger and maintain a specific and chronic inflammatory response in vitiligo patients (via IFN-γ → CXCL10 → $CD8^+$ T cells) (Laddha et al., 2013; Xie et al., 2016). The increase of this pro-inflammatory state, in turn, also maintains OS and disables the EAS in a loop process that will remain active indefinitely, inducing apoptosis of melanocytes without its proper replacement leading to vitiligo scars and disease

progression through time (Ortona et al., 2014; Lotti et al., 2015; Xie et al., 2016). Along with this metabolic profile in vitiligo patients, a lifestyle whose etiological factors are also based on low-chronic inflammation, OS, and inefficient EAS lead to the development of disorders within MetS scope and worsen vitiligo's condition.

To stop vitiligo progression and promote a favorable environment for repigmentation (maturation of melanocytes), is necessary an intervention that reduces ROS production, improves the EAS, and reduces the pro-inflammatory profile (Lotti et al., 2015). The use of antioxidants or immunosuppressant drugs to reduce ROS production is already widely used in clinical practice (Bergqvist and Ezzedine, 2020). However, these therapeutic strategies do not treat the source of ROS, being a temporary solution that does not prevent vitiligo reappearance. Further, there is no proposed intervention to improve EAS in vitiligo patients. In item 2.2, we will discuss how OS is established in vitiligo patients and how they trigger the IS response to melanocytes. We will use this information to suggest the structured physical training program (item 3) to reduce the OS condition, improve EAS, and modulate the IS.

Origin of Oxidative Stress and Vulnerable Antioxidant System: Mechanistic Factors in Vitiligo Development

It is well known in vitiligo patients that dramatic imbalance between the oxidant system (high) and EAS (deficient) leads to a predominant state of OS (Schallreuter, 2014). A higher rate of lipid peroxidation (LP) exists in individuals with vitiligo compared to healthy counterparts or generalized vitiligo compared to individuals with localized vitiligo; also, individuals with active vitiligo compared to individuals with stable vitiligo has a higher LP (Laddha et al., 2013, 2014). Thus, a greater OS results in more aggressive and severe vitiligo disease for these patients. As presented in **Figure 2**, the magnitude of ROS production in melanocytes in vitiligo patients is due to a high electrical potential in mitochondrial intermembrane space (Dell'Anna et al., 2017). These individuals have an altered mETC with a reduced distribution of cardiolipin, which leads to a defective complex I formation (Dell'Anna et al., 2017). Cardiolipins are responsible for configuring and forming mETC complexes and supercomplexes assembly (Schlame and Greenberg, 2017). Complex I is the rate-limiting step of aerobic respiration and has a central role in energy production for metabolism (Sharma et al., 2009). Therefore, disturbance in cardiolipin concentrations or distribution will lead mitochondria to inefficient energy production.

Dell'Anna et al. (2007) showed that cardiolipin concentration and distribution disturbances lead to inefficient mitochondrial melanocytes' ATP production, low glucose consumption, and high ROS emission (Dell'Anna et al., 2007). Recently, another research group (Martins et al., 2021) also showed that vitiligo skin T cells induce melanocytes to produce higher oxygen consumption and ROS emission without increasing the glycolysis flux. In their experiments, the melanocytes' ROS production was blunted when N-acetylcysteine was used as an antioxidant in the cell culture. More importantly, the excess of oxygen consumption and ROS production was blunted when ruxolitinib, a Janus kinase (jak)1/2 inhibitor, was administered in the cell culture. Taken together, these data indicated that vitiligo skin T cells induce melanocytes to increase oxygen consumption to produce ROS, overwhelming its antioxidant system. Interestingly, the lack of change in glycolysis flux in melanocytes interaction with vitiligo skin T cells (via jak/STAT signaling described in **Figure 1A**) suggests that ROS production (with the extra oxygen consumption) over to ATP production (as illustrated in **Figure 2**), as discussed in this paper, is a profile to activating cell apoptosis in epidermal from vitiligo patients.

The major ROS product found in vitiligo patients is hydrogen peroxide (H_2O_2) which plays a crucial role in LP and in disease development (Schallreuter, 2014). The enzymatic complex superoxide dismutase (SOD) and Reduced nicotinamide adenine dinucleotide phosphate- oxidase (NADPH-oxidase, specifically, the NOX4 isoform localized in the mitochondria) have been identified as the main source of H_2O_2 (Laddha et al., 2013; Barygina et al., 2015). The SOD complex has a high gene expression and enzymatic activity in individuals with active vitiligo (Glassman, 2014), probably due to elevated ROS emission from defective mitochondria (Dell'Anna et al., 2010). Elevated H_2O_2 production by SOD activity leads to an early inhibition of EAS enzymes activity such as TrxR (Schallreuter et al., 1991a), CAT and GPx, which in turn, leads to an increase in Fenton reaction, increasing hydroxyl concentrations ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH$) to a toxic level, that ultimately, leads to a significant peroxidation in several cellular components (membranes, proteins, mitochondria, and DNA) (Schallreuter, 2014). To remove H_2O_2 excess, GPx, TrxR, and CAT (i.e., enzymes involved in EAS) are dependent on reduced nicotinamide adenine dinucleotide phosphate (NAPDH) for the renewal of its substrate, thereby enabling the clearance of ROS. However, the NADPH-oxidase activity (which also uses NADPH as a cofactor) is increased (producing H_2O_2) during active vitiligo, therefore increasing ROS and promoting an active competition with EAS enzymes for NADPH molecules, a molecule already reduced in active vitiligo patients (Shajil and Begum, 2006). Thus, with the significant increases in H_2O_2 concentrations, the NADPH binding site is inhibited with EAS enzymes (Schallreuter, 2014). Therefore, the EAS enzymes are crucial for preventing systemic OS, i.e., containing H_2O_2 extravasation from the mitochondria and their respective melanocyte cells to the other sites (Lu and Holmgren, 2014); however, EAS enzymes are deactivated due to chronic elevated H_2O_2 induced by defective mitochondria (Schallreuter, 2014). Together, these studies demonstrate that the removal of ROS in vitiligo patients is a deficient process.

The increase in H_2O_2 drastically impairs calcium metabolism in the epidermis (both cell influx and efflux, as well as the structure of L-type calcium channels) (Schallreuter, 2014). This process also causes inhibition in the ROS removal processes, regulation of melanin biosynthesis, and DNA repair via allosteric regulation of TrxR by calcium metabolism (Schallreuter et al., 1991a; Gafter et al., 1997; Schallreuter, 2014). The TrxR proper functions are calcium-dependent

(TrxR + Ca^{2+} = $TrxS_2$ + NADPH $Trx(SH)_2$ + $NADP^+$); thus, both pigmentation and ROS removal may be compromised if the melanocytes do not have a stable calcium metabolism (Schallreuter and Wood, 1991). It has been demonstrated that the areas of the epidermis affected by vitiligo have a poor calcium metabolism, with low calcium uptake by melanocytes or an absence of L-type calcium channels (Schallreuter-Wood et al., 1996; Schallreuter et al., 2012). With dysfunctional calcium metabolism, patients with vitiligo also have low melatonin, serotonin (consequently, increased tryptophan), and high noradrenaline concentrations in the epidermis, which further increases the OS (Schallreuter, 2014).

The absence of calcium in the mitochondria due to chronic higher H_2O_2 production causes mitochondrial swelling (Schallreuter, 2014; Tsai et al., 2014; Ding et al., 2015). Notably, melanocytes from vitiligo patients are known to have altered mitochondria morphology that does not undergo the mitophagy process. These mitochondria are swollen with obscure and vacuolated ridges, especially in individuals with active vitiligo (Ding et al., 2015). These mitochondrial morphological changes may be related to high gene expression and the ability to stimulate the activity of the P53 tumor suppressor protein (Teulings et al., 2013) and SOD enzymes (Laddha et al., 2013); and also to suppress the mitophagy process in this pathology (Lionaki et al., 2015). The lack of melanocytes' mitophagy process suggests that highly damaged mitochondria continue to produce high amounts of ROS, which will lead cell to apoptosis (**Figure 3**). Collectively, these studies suggest that chronic high H_2O_2 concentration, *per se*, disable EAS enzymes, calcium metabolism and inhibits the mitophagy process in melanocytes; in turn, these mechanisms lead to melanocytes destruction (via IS stimulation) and to the impossibility of their maturation and resynthesis.

In summary, this evidence suggests that the origin of excess ROS production is the result of (1) defective mitochondria (due to the abnormal and reduced distribution of cardiolipins) with high intermembrane electrical potential and (2) reduced mitophagy process (due to high expression and activity of P53 protein). Furthermore, there appears to be a vulnerability or depletion of EAS enzymes to limit the excess ROS produced by the defective's mitochondria.

Reciprocal Action of Oxidative Stress and Immune System in Vitiligo

Laddha et al. (2014) verified for the first time the relationship between OS and IS in the development of vitiligo and found that melanocytes ROS overproduction precedes the immune response. Vitiligo melanocytes' have a vulnerable EAS, as previously discussed, its chronic exposure to OS promotes the increase of several autoantigen markers (DAMPs), such as the exposure of calreticulin on the cell surface and the secretion of Hsp70 to the extracellular matrix triggering a IS response (Zhang et al., 2014; Xie et al., 2016). An animal model study has been shown that Hsp70 secretion by melanocytes induces the progression of vitiligo (Mosenson, 2013). Extracellular Hsp70 is a potent inducer of the innate and adaptive immune response. For instance, it induces the release of cytokines such as TNF-α, IL-1β, IL-6, and IL-12. In contrast, intracellular Hsp70 has a cytoprotective, anti-apoptotic, and anti-inflammatory role (Asea, 2007). Situations of psychological stress or trauma (cellular damage), pre-apoptotic cells (necrosis) secrete high concentrations of IFN-γ and ROS, which stimulates Hsp70 released to the extracellular environment (Asea, 2007). This rationale is partially supported by the fact that individuals with vitiligo in the active phase develop depigmentation in areas that suffer mechanical trauma (Lee et al., 2004).

A case study showed that the increase in IFN-γ (as a form of treatment for other pathologies) caused the appearance of vitiligo (Kocer et al., 2009), and treatment with anti-IFN-γ significantly decreased vitiligo progression (Skurkovich and Skurkovich, 2006). Both in humans and in rats, IFN-γ modulates

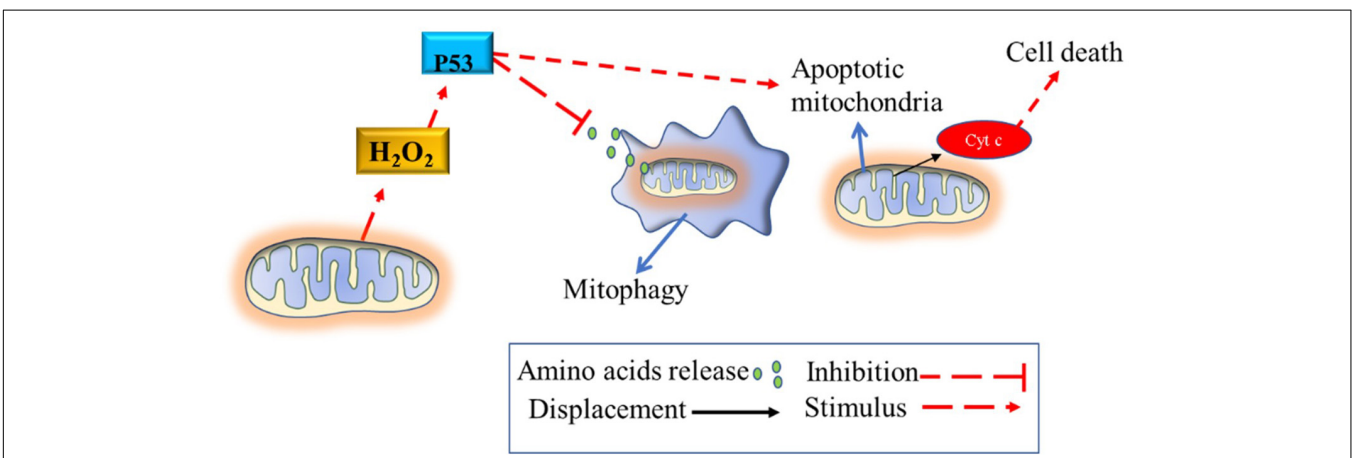

FIGURE 3 | Blunted mitophagy in melanocytes of vitiligo patients. The mitophagy process of defective mitochondria is inhibited by the high activity of P53, which is stimulated by the high production of hydrogen peroxide. Thus, defective mitochondria continue to produce reactive oxygen species, causing oxidative stress until the cellular apoptosis process is induced via mitochondrial cytochrome c release or via the cytotoxic T cell pathway recruitment (described in **Figure 1A**). The process of mitophagy destroys defective mitochondria releasing amino acids into the cellular cytosol that can be used for other cellular functions. H_2O_2, hydrogen peroxide; p53, tumor suppressor p53.

the pigmentation state of melanocytes, but in high concentrations limits melanocyte's maturation and differentiation (Natarajan et al., 2014). Dwivedi et al. (2013) showed that in patients with active vitiligo (appearance of recent spots), there is a higher IFN-γ gene expression than in individuals without vitiligo or with stable vitiligo (individuals without the occurrence of new spots in the last six months). Furthermore, in animal models (rats) and in perilesional skin from vitiligo patients, the increase in IFN-γ, IFN-α, TNF-α, cytotoxic granulation marker (CD107a) (You et al., 2013; Bertolotti et al., 2014) were positively correlated with $CD8^+$ T cells activation in melanocytes and with vitiligo severity (melanocytes destruction without their replacement). This evidence suggests a feedback loop between innate and adaptative immune responses in melanocyte's skin in active vitiligo. As previously referred, this loop is fed by melanocytes OS, which leads melanocytes to secret DAMPs and stimulating IFN-γ production, a pivotal cytokine to recruit and guide recirculating $CD8^+$ T cells to melanocytes via IFN-γ–CXCL9/10–CXCR3 axis (**Figure 1**).

Elevated concentrations of specific melanocytes $CD8^+$ memory T cells (secreting high IFN-γ) were found in vitiligo patients, mainly during active disease state (Riding and Harris, 2019). The maintenance of memory $CD8^+$ T cells in depigmented areas prevents repigmentation and is responsible for the disease recurrence after treatment interruption. It has been described that the progression and recurrence of vitiligo occur when memory $CD8^+$ T cells residing in the epidermis secrete IFN- γ (in response to DAMPs from melanocytes), which induces the recruitment of recirculating memory $CD8^+$ T cells (Riding and Harris, 2019). It is important to refer that this mechanism of melanocytes apoptosis seems to occur in a low rate of Tregs/$CD8^+$ T cells, i.e., insufficient Tregs suppressing melanocyte-specific $CD8^+$ T cells, putting in anergy state or suppressing (Riding and Harris, 2019). It has been suggested that successful treatments need to neutralize the activity of recirculating memory $CD8^+$ T cells or memory cells residing in melanocytes (e.g., by immunosuppression) to prevent disease progression (Rashighi et al., 2014; Riding and Harris, 2019).

Vitiligo patients can be characterized by a pro-inflammatory profile with high expression of T helper (Th) 1 and Th17 populations and low expression of Treg and Th2 populations. The literature reports a higher concentration of inflammatory cytokines markers such as IL-2, IL-6, IL-15, TNF-α, and IFN- γ and, in contrast, decreased anti-inflammatory cytokines like IL-4 and IL-10 (Lotti et al., 2015; Riding and Harris, 2019).

Overall, continued OS production can sustain chronic inflammation indefinitely (Lotti et al., 2015). This chronic inflammation plays a key role in the repigmentation process of vitiligo wounds (Lotti et al., 2015). It has been long known that the repigmentation process of the affected areas needs the migration and proliferation of immature melanocytes as well as the reestablishment of calcium metabolism. However, these cell renewal processes do not occur when high levels of pro-inflammatory cytokines and/or OS are predominant in depigmented areas (Schallreuter, 2014). Taken together, an effective treatment to limit vitiligo progression and recurrence needs to improve i) the EAS enzymes (i.e., GPx, TrxR, and CAT); ii) the function and quality of mitochondria structure in melanocytes and $CD8^+$ T cells; and iii) change the profile of the immune system.

POTENTIAL ROLE OF CHRONIC PHYSICAL EXERCISE ON VITILIGO DEVELOPMENT

Chronic PE has great potential to improve EAS (consequently decreasing OS), mitochondrial function and decrease the inflammatory profile (**Table 1**). Hypothetically, this modulation can prevent vitiligo progression and probably facilitate vitiligo wound healing. Nonetheless, to date, there are no studies analyzing the clinical applicability of PE training or physical activity level in vitiligo disease. Therefore, based on state-of-the-art evidence from PE literature (redox system, mitochondrial function/structure, and immunology), we will propose *PE* as a potential therapeutic strategy on vitiligo disease as summarized in **Table 1**.

Potential Role of Chronic Physical Exercise on Redox System Modulation: Implications on Vitiligo Development

Vitiligo Patients have an associated high rate of inflammatory comorbidities such as MetS and cardiovascular diseases (Lotti and D'Erme, 2014). These diseases are also etiologically related to a higher OS. Notably, accumulating evidence has shown that structured chronic PE training or high cardiorespiratory fitness (CRF) decreases the risk of OS-related pathologies (Farrell et al., 2012; de Sousa et al., 2016; Lu et al., 2021).

Initially, acute PE (mainly the aerobic-type) was believed to be a bad approach once it increases 20 times oxygen consumption and sharply increases ROS and reactive nitrogen species (RNS) production (Leeuwenburgh and Heinecke, 2001). Paradoxically, chronic PE was known to induce a positive decrease in ROS production during acute exercise and at rest (Leeuwenburgh and Heinecke, 2001). Today, it is well established that skeletal muscle ROS produced during acute PE is responsible for several positive adaptations in skeletal muscle and other tissues. Each bout of PE (resistance or aerobic-type) induces putative ROS/RNS homeostasis, which is necessary to induce gene translation related to aerobic metabolism (e.g., mitochondrial proteins) and to muscle protein synthesis (e.g., EAS enzymes) (Carlos Henríquez-Olguín et al., 2019; Vargas-Mendoza et al., 2019). Therefore, the well-established concept that individuals with high CRF have low ROS levels (when compared to individuals with low CRF) is a direct result of acute ROS stimulation by PE bouts. For instance, improved mitochondrial function due to aerobic training results in decreased mitochondrial electrical potential. In physically trained individuals, OS becomes smaller both at rest and during acute PE when compared with sedentary individuals (Venditti et al., 1999). Further, the hormetic effect of chronic PE (Radak et al., 2014; Margaritelis et al., 2018; Aguiar et al., 2021) is an efficient tool to promote improvement in EAS, especially in susceptible individuals to a greater situation of OS

TABLE 1 | The potential role of physical exercise training on vitiligo patients.

Modifiable vitiligo profile	Potential from acute exercise	Potential from physical training
Metabolic profile		
It is associated to MetS and ↑ insulin resistance, adipose tissue, blood pressure, LDL-C, and ↓ HDL-C.	↑ gene transcription to glucose uptake, fat oxidation, cardiac and vascular remodeling.	↓ insulin resistance. ↓ LDL-C and blood pressure. ↑HDL-C. Improvement in several MetS markers. ↓ adipose tissue.
Redox system profile		
↑ ROS/RNS. ↓ EAS enzyme (GPx, TrxR, and CAT). Chronic ↑ NADPH-oxidase and SOD activity. ↑Lipid, protein, and DNA peroxidation.	Acute ↑ NADPH-oxidase activity induces EAS enzymes gene transcription (via NF-κB pathway). ↑ Nrf2-ARE/HO-1 pathway activation.	↑ EAS enzymes capacity and ↓ lipid, protein, and DNA peroxidation. ↓ROS. ↓ NADPH-oxidase activity-induced ROS. ↑ NADPH synthesis.
Mitochondrial structure and function profile		
↓ mitochondrial mitophagy in melanocytes. ↓ cardiolipin quality and quantity; ↓ melanocytes mitochondrial ATP production and mitochondrial complexes and supercomplex activity. ↑ mitochondrial ROS emission.	↑ gene transcription for mitochondrial biogenesis and remodeling. ↑ IGF-1/PI3K/AKT/ACL pathway activation to cardiolipin biosynthesis.	↑ mitochondrial mitophagy and remodeling (mitofision, mitofusion). ↑ ATP mitochondrial (increase in complex 1) and mitochondrial mass. ↑ cardiolipin content and supercomplex formation and ATP content. ↓ mitochondrial ROS emission.
Immune function profile		
↑ IL-2, IL-6, and IL-15. ↓ IL-4 and IL-10. ↑ TNF-α, IFNα, and IFN- γ. ↑ memory $CD8^+$ T cell. ↓Tregs. ↑ extracellular HSP.	$CD8^+$ T cells mobilization to bloodstream removing hyper-reactive senescent cells. ↑ acute increase in IFN-α, IL-6, and IL-1β inducing a regulatory effect in IL-4, IL-10, and IL-1RA.	↓ IL-2, IL-6, and ↑ IL-15 ↑ IL-10 and IL-4. ↓ TNF-α and IFN- γ. ↓ total lymphocytes, $CD8^+$ T cell proliferation, memory $CD8^+$ T cell, and ↑ senescent $CD8^+$ T cell apoptosis. ↑ Tregs. ↓ extracellular HSP and ↑ intracellular HSP

*References from this table are provided as **Supplementary Material**. ↑, increase; ↓, decrease; ATP, adenosine triphosphate; CAT, catalase; EAS, endogenous antioxidant system; GPx, glutathione peroxidase; HSP, heat shock protein; HDL-C, high-density lipoprotein- cholesterol; HSP, heat shock protein; IFN, interferon; IGF-1, insulin-like growth factor 1; IL, interleukin; LP, lipid peroxidation; LDL-C, low-density lipoprotein- cholesterol; MetS, metabolic syndrome; Nrf2-ARE/HO-1, Nuclear Factor E2 related to Factor 2-antioxidant/heme oxygenase 1 response element; ROS/RNS, reactive oxygen species/reactive nitrogen species; SOD, superoxide dismutase; TNF, tumor necrose factor; Tregs, regulatory T cells; TrxR, thioredoxin reductase.*

such as obesity, type 2 diabetes (T2D) or with cardiovascular disease (Radak et al., 2014; de Sousa et al., 2016). Even in healthy individuals, chronic PE (especially aerobic types) leads to redox system adaptations that decrease OS levels (Margaritelis et al., 2018; Aguiar et al., 2021). Finally, it is important to mention the conclusions of a meta-analysis (de Sousa et al., 2016; Aguiar et al., 2021), supporting that chronic PE tends to decrease OS markers and increase the antioxidant system, without a redox imbalance after a well-designed PE program.

An elegant study (Gifford et al., 2016) showed that individuals with high CRF (VO_2max, ∼59 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$) have high amounts of mitochondria (i.e., "excess"), and are far from saturating mitochondrial work capacity even in high-intensity PE. In contrast, in individuals with low CRF (VO_2max, ∼38 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$) easily exceeded mitochondrial work capacity. According to the authors (Gifford et al., 2016), this mitochondrial "excess" or reserve mitochondria can work as ROS removal system, thus, preventing mitochondria's mass from reaching its maximal respiratory capacity, which can damage their structures (Sansbury et al., 2011).

Vitiligo patients have a deficient endogenous EAS with low CAT activity (Maresca et al., 1997) both in the epidermis (Schallreuter et al., 1991b) and in the blood plasma (Shajil and Begum, 2006). In addition, GPx and TrxR have low activity in vitiligo patients (Schallreuter et al., 1991b; Shajil and Begum, 2006). The nuclear factor E2 related to factor 2-antioxidant/heme oxygenase 1 response element (Nrf2-ARE/HO-1), a key metabolic pathway for genetic signaling transduction of enzymes related to the antioxidant system, is impaired in vitiligo patients (Jian et al., 2014). The upregulation of the Nrf2-ARE/HO-1 axis is necessary to improve melanocytes' tolerance to ROS stressors (mainly from H_2O_2). Notably, if the Nrf2-ARE/HO-1 axis metabolic pathway is stimulated in melanocytes, the ability to remove ROS in these cells is restored (Jian et al., 2014). Further, animal studies have shown that there is an increase in Nrf2 expression, increasing the expression of antioxidant enzymes after acute (Muthusamy et al., 2012) and chronic PE (Gounder et al., 2012).

Acute PE also increases signaling pathways of the enzymatic antioxidant system by NADPH-oxidase→ NF-κB (Carlos Henríquez-Olguín et al., 2019). Although NADPH-oxidase activity during acute PE is the main source of ROS, this increase in ROS is necessary to induce GPx, CAT and SOD enzymes gene expression as well as mitochondrial biogenesis via

NF-κB (Henríquez-Olguín et al., 2016). On the other hand, in animal models, chronic aerobic-exercise training reduces ROS produced by NADPH-oxidase isoforms (NOX2 and NOX4) (Qi et al., 2020). However, more studies are needed to determine if chronic PE training decreases NADPH-oxidase isoforms (i.e., NOX4) activity from immune cells infiltrated in melanocytes from vitiligo patients (Barygina et al., 2015). Moreover, as the decrease in NADPH-oxidase activity results in reduced ROS levels emission (Adams et al., 2005) more studies are also needed to verify if the decrease in ROS production induced by chronic PE has clinical significance in vitiligo patients.

Another mechanism by which chronic PE can improve ROS production in vitiligo disease is the increase of NADPH availability (a molecule with reduced concentrations in vitiligo patients) (Shajil and Begum, 2006). The decrease in NADPH-oxidase activity in response to chronic PE can increase the availability of NADPH for glutathione reductase to renew GSH and TRx (SH)2 (Jenkins and Goldfarb, 1993; Pannala and Dash, 2014). So, the H_2O_2 produced by SOD (an enzyme with high activity in vitiligo) can be properly removed by GPx and TrxR (Leeuwenburgh and Heinecke, 2001; Pannala and Dash, 2014). A schematic overview of potential role of PE on redox system modulation is illustrated in **Figure 4**.

Potential Role of Chronic Physical Exercise on Immune System Modulation: Implications on Vitiligo Development

It has been well established that structured chronic PE has an anti-inflammatory effect (Gjevestad et al., 2015), especially in MetS (Alizaei Yousefabadi et al., 2020). A recent meta-analysis shows a decrease in pro-inflammatory cytokines such as IFN-γ, TNF-α, and IL-8 and increase in anti-inflammatory IL-10 after chronic PE (Alizaei Yousefabadi et al., 2020). In addition, an early systematic review pointed that chronic PE induces a decrease in Th1 cell lineage gene expression (Gjevestad et al., 2015). The Th1 gene expression is responsible for the production of IFN- γ, which induces the activation of a set of chemokines, including CXCL10, that in vitiligo patients is responsible for the chemotaxis of $CD8^+$ T cells to melanocytes and to start the process of destruction of these cells (Rashighi et al., 2014). On the other hand, it is well established the anti-inflammatory role of IL-10, which induces up-regulation of Th2 that, in turn, inhibits Th1 cytokines (for example, increases Tregs cells and downregulates IFN-γ). IL-10 also inhibits antigen presentation by APC (Abbas et al., 2019a; see **Figure 1B**).

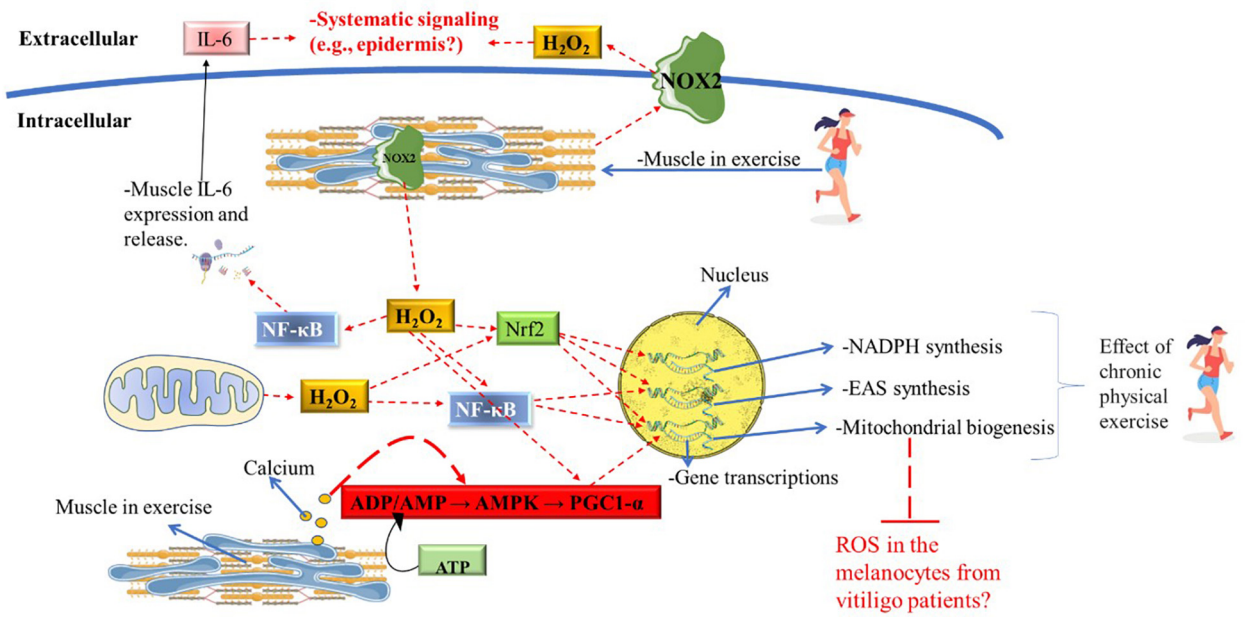

FIGURE 4 | Potential role of chronic PE on redox system in vitiligo patients. Muscle contraction and mitochondrial activity produce large amounts of hydrogen peroxide during physical exercise, acting as an activation signal for the NF-κB, Nrf2, and, PGC1-α metabolic pathway downstream. ATP degradation and calcium release caused by muscle contraction also significantly induce the AMP/AMPK/PGC1-α axis. During acute exercise, gene transcription of EAS enzymes is significantly induced by the NF-κB and Nrf2o pathways; genes encode enzymes in the pentose phosphate pathway (i.e., for NADPH synthesis) is induced by the Nrf2 pathway; mitochondrial gene transcription is induced by the NF-κB, Nrf2, and PGC1-α pathways. Only chronic physical exercise will significantly induce the synthesis of mitochondria, EAS enzymes, and NADPH synthesis. Muscle contraction also releases a large amount of IL-6 and hydrogen peroxide to the extracellular medium, which may exert systemic signaling. Future research is needed to identify whether IL-6 and hydrogen peroxide from muscle tissue can exert significant signaling in the epidermis. Also, further research will be needed to assess whether the increase in mitochondria in muscle tissue can buffer the ROS produced by melanocytes in the epidermis of patients with vitiligo and whether this has clinical relevance. ADP, adenosine diphosphate; AMP, adenosine monophosphate; AMPK, adenosine monophosphate-activated protein kinase; ATP, adenosine triphosphate; H_2O_2, hydrogen peroxide; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, Nuclear factor-erythroid factor 2-related factor 2; IL-6, interleukin-6; NOX2, NADPH oxidase isoform 2; PE, physical exercise; ROS, reactive oxygen species.

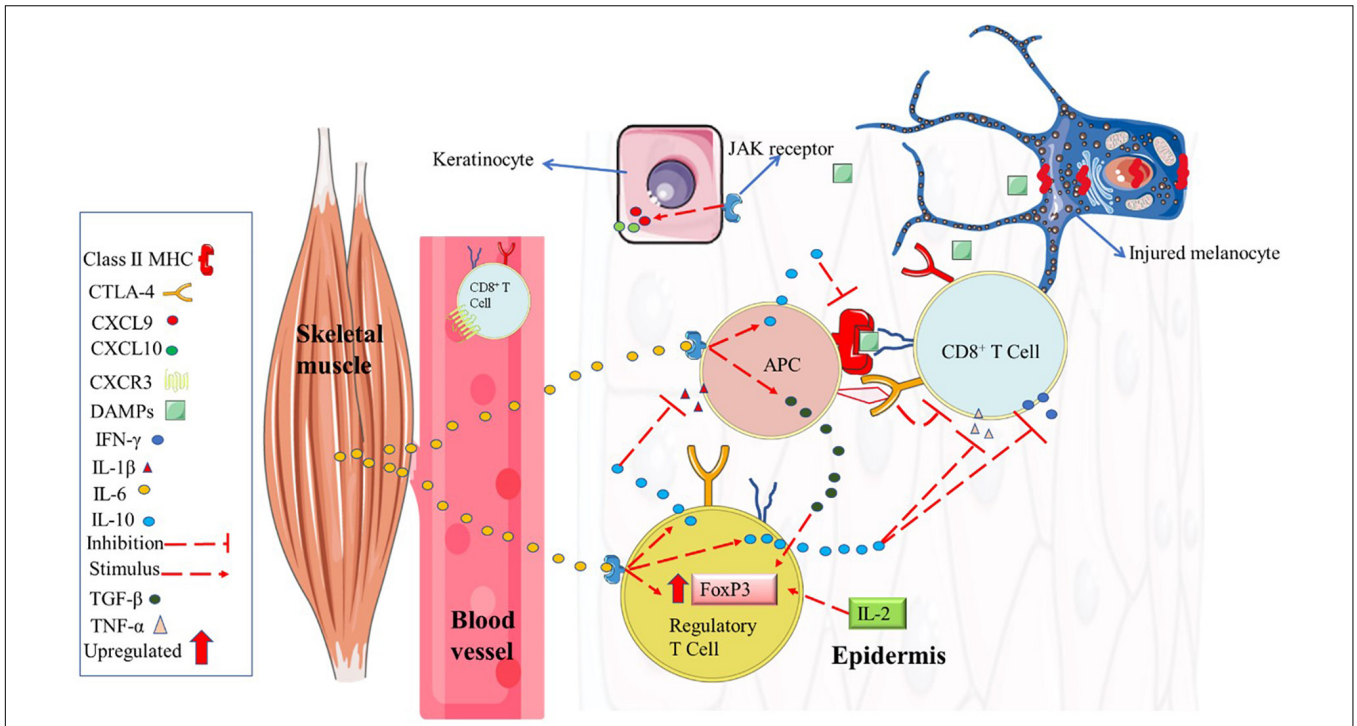

FIGURE 5 | Potential role of chronic physical exercise on immune system modulation in vitiligo patients. Physical exercise can induce skeletal muscle to release huge amounts of IL-6, which has the potential to reach the skin and induce a local adaptation of the immune system (anti-inflammatory profile). For example, hypothetically high amounts of IL-6 in the skin can be detected by skin-resident APC and regulatory T cells that, in turn, give negative feedback releasing IL-10. IL-10 can inhibit the local production of IL-1, IFN-γ, and TNF-α. Inhibiting IL-1-β and TNF-α, there is no negative feedback for CTLA-4 expression in cytotoxic T cells (the interaction between CTL-4 from $CD8^+$ T cell with costimulatory molecule B7 from APC suppress $CD8^+$ T cell activation). Also, IL-10 could inhibit $CD8^+$ T cells by inhibiting the expression of costimulatory class II MHC molecules from APC. IL-6 also induces significant TGF-β expression, resulting in upregulation of FoxP3. APC, antigen-presenting cell; CD28, Cluster of Differentiation 28; CTLA-4, cytotoxic T lymphocyte antigen-4; CXCL, C-X-C motif chemokine ligand; CXCR, C-X-C motif chemokine receptor; DAMPs, damage-associated molecular pattern; JAK/STAT, Janus kinase/signal transducer and activator of transcription; IFN-γ, interferon-gamma; IL, interleukin; MHC, major histocompatibility complex; TGF-β, transforming growth factor, TNF-α, tumor necrosis factor-alpha. Figure created with images from smart.servier.com.

It is well known that the main anti-inflammatory effect from chronic PE derives from the acute large release of muscle IL-6 (during acute exercise bouts) to the bloodstream, which exerts a paracrine effect in several tissues and organs such as the brain, bones, and digestive tract (Ellingsgaard et al., 2019). IL-6 from muscle tissues stimulated by acute PE modulates the Treg and Th17 cells ratio via IL-10 (**Figure 5**). This process decreases IS autoreactive potential by increasing Treg and decreasing Th17 ratio, which is a process opposite to vitiligo onset (Lotti et al., 2015; Xie et al., 2016). Also, it appears that muscle release of IL-6 plays an important role in the PBMCs hypomethylation (Horsburgh et al., 2015).

The decrease in Th1 and Th17 expression, and the increase in Treg and Th2 population, is also related to a powerful effect that chronic PE exerts on Hsp70. It was recently shown that acute PE increases extracellular Hsp70 concentrations (more specifically in blood plasma); however, it was followed by a concomitant increase in intracellular Hsp70 concentration (more specifically in peripheral blood mononuclear cells- PBMCs) (Lee et al., 2015). As a chronic effect of PE training on Hsp70 in PBMCs, there is an increase in intracellular at basal levels associated with their decrease in extracellular sites (Périard et al., 2016).

Also, acute PE mobilizes high concentrations of $CD8^+$ T cells (part of them from the skin) in response to the increase in catecholamines during its practice (Turner et al., 2016). This IS cells mobilization into the bloodstream during PE plays a significant role in removing hyper-reactive IS cells (Krüger and Mooren, 2014). For example, a session of high-intensity resistance exercise (60% 1RM; each set to concentric failure) is enough to induce temporary immunosuppression (i.e., a reduction in the ratio of CD4:CD8 T cells below 1:1; when the normal value is 1:4) (Jin et al., 2015). Also, 6-weeks (3x/week; 8–10 sets of 60s at 100% VO_2peak) of high-intensity interval training (HIIT) can promote an anti-inflammatory state, attenuating the proliferation of a subset $CD8^+$ T cell ($CD8^{low}$, which produces high levels of IFN- γ and TNF-α) (Shiu, 2016). In addition, eight weeks of HIIT (5km running; work: rest ratio 1:1 ratio, 1 min. at 100% vVO_2max interspersed with 1 min. of passive recovery) plus resistance training (4 sets of squats at 80% 1RM to concentric failure) promoted an increase in IL-10 and IL-6 (Monteiro et al., 2017). These studies suggest that if this training routine (high volume or high intensity) is sustained for weeks, it will induce a sustained IS alteration, triggering a state of temporary immunosuppression in response

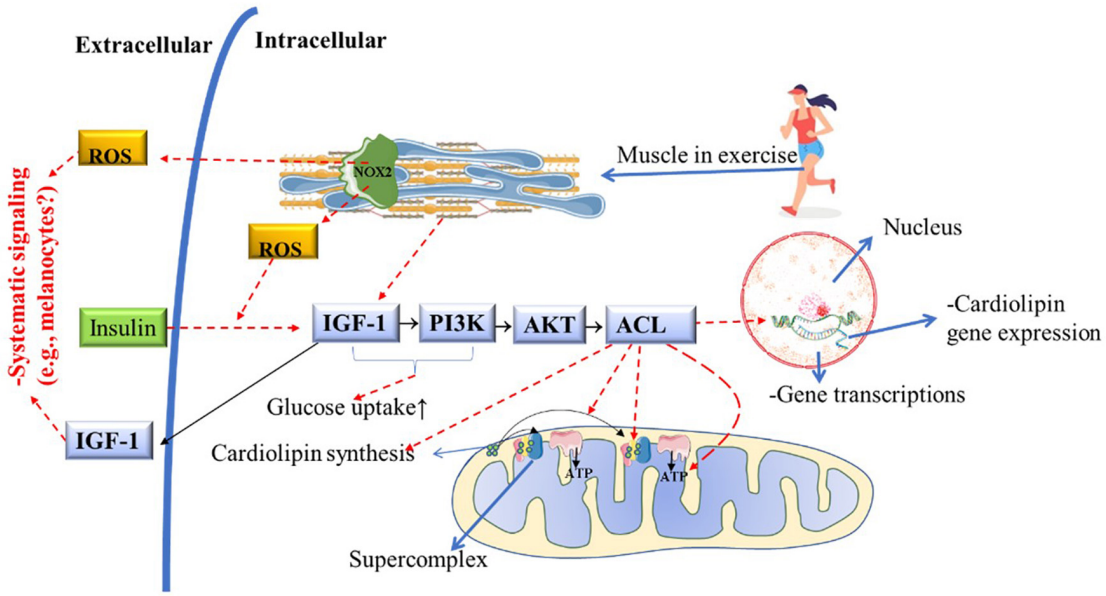

FIGURE 6 | Potential role of chronic PE on cardiolipin metabolism, mitochondrial function, and structure in vitiligo patients. Muscle contraction during acute physical exercise activates and releases large amounts of IGF-1 into the bloodstream. Muscle contraction or insulin action is required to activate IGF-1 and the PI3K/AKT/ACL downstream metabolic pathway. ACL activation is necessary for cardiolipin synthesis and gene transcription. As a consequence of ACL activity, mitochondria increase the mitochondrial complexes and supercomplexes content and activity, enhancing ATP production (i.e., improving the Mitochondrial structure and function). Nox2 activation by acute physical exercise improves insulin-sensitive, increasing GLUT-4 translocation to the cellular membrane). This hypothetically facilitates the IGF-1 metabolic pathway downstream, consequently improving mitochondrial structure and function and preventing insulin resistance. ACL, Adenosine triphosphate-citrate lyase; ADP, adenosine diphosphate; AKT, protein kinase; GLUT4, glucose transport 4; IGF1, insulin-like growth factor-1; NOX2, NADPH oxidase isoform 2; PE, physical exercise; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species.

to this type of PE training (Shaw et al., 2017). Theoretically, this immunosuppression state imposed by high-load chronic PE can be seen as a protective factor to reduce the risk of developing autoimmune diseases (Shiu, 2016).

An interesting study observed that lifelong aerobic-trained individuals (with 40 $ml \cdot kg^{-1} \cdot min^{-1}$ VO_2max) had higher blood Tregs markers concentrations (IL-10, forkhead box P3, and transforming growth factor-β), low ↓TNF-α/IL-10↑ ratio associated with lower memory $CD8^+$ T cells when compared to sedentary counterparts (with 29 $ml \cdot kg^{-1} \cdot min^{-1}$ VO_2max) (Minuzzi et al., 2017, 2018). Furthermore, IL-2, which is responsible for T cell proliferation and differentiation, and maturation of $CD8^+$ memory T cells is decreased after chronic PE (Rhind et al., 1996). Notably, vitiligo patients have high IL-2 concentrations due to a high stimulation of T cells (Jian et al., 2014); and lower Tregs markers (IL-10 and forkhead box P3) (Lotti et al., 2015; Bhardwaj et al., 2020). A theoretical schematic model of the potential role of chronic PE on IS from vitiligo patients is pointed out in **Figures 1B**, **5**.

Potential Role of Chronic Physical Exercise on Mitochondrial Function and Structure: Implications on Vitiligo Development

Melanocytes and PBMCs from vitiligo patients have deficient energy metabolism due to the low concentration and abnormal distribution of cardiolipin in the mETC, which prevents the stability and creation of supercomplexes (Dell'Anna et al., 2007). Due to decreased cardiolipin metabolism, melanocytes from vitiligo patients have low energy production from glycolytic phosphorylation (due to defects in complex 1), resulting in low ATP production and high mitochondrial ROS production (**Figure 2**; Dell'Anna et al., 2017). Recently it was demonstrated that skeletal muscle increases mitochondrial cardiolipin quality and concentrations in response to IGF-1/PI3K/AKT/ACL pathway activation, which is induced by chronic physical aerobic exercise (**Figure 6**; Das et al., 2015). Interestingly, HIIT is a potent inducer of complex 1 enhancement in skeletal muscle (Bishop et al., 2014), leading to a greater mitochondrial ATP production and increase carbohydrate phosphorylation (Nilsson et al., 2019), which is impaired in melanocytes from vitiligo patients (Dell'Anna et al., 2017). On the other hand, in skeletal muscle, continuous aerobic-exercise training increases mitochondrial tissue volume (Bishop et al., 2014), which is a morphological change important to improve ROS clearance (Sansbury et al., 2011; Gifford et al., 2016).

In animal models developing T2D, a drastic decrease in cardiolipin levels in striated muscle was identified (Han et al., 2007). Therefore, hypothetically, insulin resistance (or T2D) may increase the risk of vitiligo developing in genetically predisposed individuals. In **Figure 6**, it is shown that PE improves insulin sensitivity via NOX2 (Carlos Henríquez-Olguín et al., 2019), and consequently, insulin signaling increases IGF-1activation

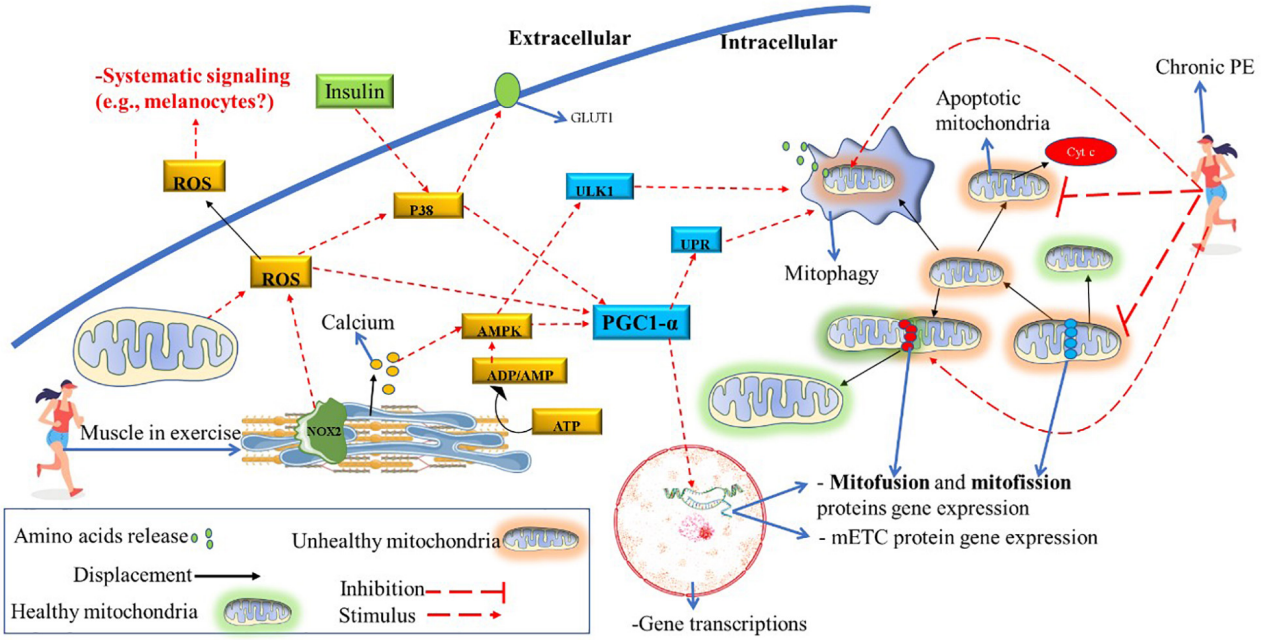

FIGURE 7 | Potential role of chronic PE on mitochondrial function and structure. ATP degradation and calcium release caused by muscle contraction also significantly induce the PGC1-α and ULK1 downstream pathways. Also, muscle contraction stimulates large ROS released (by NOX2 and mitochondria activity), further activating the PGC1-α downstream. Acute PGC1-α activation results in gene transcription related to mitochondrial remodeling (mitofusin, mitofission, and mETC proteins) and mitophagy induction. Chronic PE will decrease the mitofission pathway and significantly enhance the mitofusion and mitophagy pathways. Consequently, the mitochondrial apoptosis pathway, which can induce cell death (due to the release of cytochrome C), decreases due to chronic PE. Acute PE also activates P38, an important stimulator of PGC1-α. For example, the lack of activity of this protein can decrease PGC1-α activation, even when there are other stimuli (e.g., only AMPK stimulation). Also, P38 is activated by insulin; in turn, p38 increases cell glucose uptake upregulating GLUT1. Hypothetically, a sedentary lifestyle and insulin resistance decrease the pulsative activation of p38, thus decreasing mitochondrial remodeling potential. ADP, adenosine diphosphate; AMP, adenosine monophosphate; AMPK, adenosine monophosphate-activated protein kinase; ATP, adenosine triphosphate; GLUT1, glucose transport 1; mETC, mitochondrial electron transport chain; NOX2, NADPH oxidase isoform 2; p38, mitogen-activated protein kinase; PE, physical exercise; PGC1-α, peroxisome proliferator-activated receptor γ coactivator-1α; ROS, reactive oxygen species; ULK1, Unc-51 Like Autophagy Activating Kinase 1; UPR, unfolded protein response.

(Das et al., 2015). It is well-known that chronic PE (HIIT) can reverse insulin resistance and the development of T2D and improve muscle mitochondrial capacity (Little et al., 2011). Therefore, future studies should investigate the relationship between insulin resistance and vitiligo development (if it is a cause-effect relationship or a common pathway). Also, the potential benefits of PE in improving insulin sensitivity and the IGF-1/PI3K/AKT/ACL metabolic pathway in vitiligo patients deserve further investigation. Moreover, both insulin and PE also activates P38 in skeletal muscle, an vital molecule to activate PGC1-α downstream pathway (see **Figure 7**) to promote mitochondrial remodeling resulting in improvement in glucose and fat metabolism (Hood et al., 2015). The pulsative activation of p38 in skeletal muscle is required for whole-body energy expenditures, which can prevent obesity and D2T (Bengal et al., 2020). However, P38 has a pleiotropic effect and opposite effect in different cell lines and cell environment (Stramucci et al., 2018). For instance, chronic high H_2O_2 levels can activate p38 continuously, inducing melanocytes to premature senescence (dysfunctional) and susceptible to apoptosis (Hou et al., 2022). The same pattern is found in skeletal muscle, the continuous p38 activation induces proinflammatory cytokines expression, insulin resistance (GLUT4 downregulation) and pathological muscle atrophy (Bengal et al., 2020). Therefore, further studies is needed to determine the role of chorionic PE on P38 activation and signaling in melanocyte of vitiligo development.

Vitiligo patients have a reduced mitophagy process in epidermal melanocytes (Ding et al., 2015). It is speculated that vitiligo is a systemic pathology (Lotti and D'Erme, 2014); however, it is not known whether this condition (decreased mitophagy) extends to other tissues. In the animal model (during acute PE), the impaired mitophagy process is characterized by low resistance to endurance exercise and a high metabolic acidosis (Vainshtein et al., 2015). Therefore, we can speculate that during acute PE, vitiligo patients produce higher lactate concentrations when compared to their healthy peers (with the same CRF values). It is also well established that PE (mainly aerobic type) is a potent inductor of the mitophagy process and mitochondrial turnover/remodeling (Hood et al., 2015; Tarpey et al., 2017). As chronic PE induces tissue changes that go beyond skeletal muscle tissue (e.g., increase in mitochondria in adipose tissue), it is plausible that changes such as improvement in mitochondrial metabolism (improves their structure and function) in the epidermis also could occur in response to physical training practice (see **Figure 6**). (Also, **Table 1** summarizes the potential role of acute and chronic PE on

vitiligo disease, which deserves future research in the epidermis of vitiligo patients).

CONCLUSION AND FUTURE DIRECTIONS

To the best of our knowledge, there are no studies in the literature that analyze the clinical applicability of chronic PE as a treatment strategy to improve vitiligo adverse events. According to the evidence discussed in this paper, we can hypothesize that chronic PE can modulate the etiological factors related to the onset of this condition which involves a high and chronic ROS production, a deficient EAS enzymatic activity, and a pro-inflammatory autoimmune response (Xie et al., 2016). Further, if confirmed, the improvement in these molecular mechanisms can lead to important clinical implications in vitiligo phenotypic traits and disease prognostic, that ultimately, may improve patient's self-image, body confidence, and reduce psychological distress associated with this disease (Bonotis et al., 2016). Therefore, structured PE training may have a significant clinical impact not only in disease prognostic but also in patients' quality of life.

The literature has shown that chronic PE plays a significant role in EAS enzymatic metabolism, IS modulation, and mitochondrial quality and function. However, more research is highly required from both observational and experimental studies to investigate the role of chronic PE training in vitiligo disease on four spheres: (i) disease prevention; (ii) during active disease development; (iii) during its stable/management phase; and (iv) during the repigmentation phase.

Observational studies comparing the general population with this clinical population should verify the relationship of daily physical activity with vitiligo development or severity. For instance, studies that investigate if CRF level or daily step count is associated with vitiligo's disease. Further, experimental studies, in turn, should evaluate in more controlled settings, several aspects: (i) the role of acute and chronic PE in the *molecular mechanisms that are underlying the onset* of the disease and thus, test whether these positive changes in mitochondria, EAS enzymes and IS modulation reflects on the epidermis and in the melanocytes from vitiligo patients; (ii) test the *dose-response of different PE* types, volumes, and frequencies in vitiligo patients. Therefore, we suggest some clinically relevant markers that can be analyzed to test the potential applicability of chronic PE in vitiligo disease:

- ✓ Investigate if exercise training **improves EAS and decreases LP rates** in vitiligo patients.
- ✓ Investigate whether exercise training **improves the inflammatory profile** on vitiligo patients (i.e., the Th1/Th2 ratio), as well as extracellular Hsp70 concentrations.
- ✓ Check whether exercise training **alters the pigmentation/depigmentation process progression** in patients with vitiligo.
- ✓ Check whether the **energy metabolism** (respiratory quotient and plasma lactate concentrations) of patients with vitiligo differs from healthy peers, i.e., check whether these changes extend to muscle tissue. Currently, there are no studies associating the level of physical activity or CRF and the incidence of vitiligo in genetically susceptible individuals.
- ✓ Check whether PE training **alters the melanocytes mitochondria morphology** of vitiligo patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

EF and EC designed the study and wrote the first draft. LB, RS, MDS, VH, AF, and RM added important intellectual content writing, criticizing, and correcting previous versions of the manuscript. All authors approved the final version of the manuscript.

ACKNOWLEDGMENTS

EF is thankful to FAPESP—Fundação de Amparo à Pesquisa do Estado de São Paulo (grant no. 2021/03601-01) and CAPES—Coordenação de Aperfeiçoamento de Pessoal de Nível Superior to providing the Ph.D. and postdoctoral scholarships, respectively, which made possible to conduct such study and many other experiments.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.843784/full#supplementary-material

REFERENCES

Abbas, A. K., Lichtman, A. H., and Pillai, S. (2019a). *Immunologic Tolerance and Autoimmunity in Cellular and Molecular Immunology*, 9 Edn. Philadelphia: Elsevier.

Abbas, A. K., Lichtman, A. H., and Pillai, S. (2019b). *Innate Immunity Cellular and Molecular Immunology, Ninth Edition*. Philadelphia: Elsevier.

Adams, V., Linke, A., Kränkel, N., Erbs, S., Gielen, S., Möbius-Winkler, S., et al. (2005). Impact of regular physical activity on the NAD (P) H oxidase and angiotensin receptor system in patients with coronary artery disease. *Circulation* 111, 555–562. doi: 10.1161/01.CIR.0000154560.88933.7E

Agarwala, S., and Malkud, S. (2020). A study on the clinico-epidemiological profile of vitiligo patients and its association with endocrine, audiological and ocular abnormalities. *Iranian J. Dermatol.* 23, 155–162. doi: 10.22034/ijd.2020.120835

Aguiar, S. S., Sousa, C. V., Santos, P. A., Barbosa, L. P., Maciel, L. A., Coelho-Júnior, H. J., et al. (2021). Master athletes have longer telomeres than age-matched

non-athletes. A systematic review, meta-analysis and discussion of possible mechanisms. *Exp. Gerontol.* 146:111212. doi: 10.1016/j.exger.2020.111212

Alizaei Yousefabadi, H., Niyazi, A., Alaee, S., Fathi, M., and Mohammad Rahimi, G. R. (2020). Anti-inflammatory effects of exercise on metabolic syndrome patients: a systematic review and meta-analysis. *Biol. Res. Nurs.* 23, 280–292. doi: 10.1177/1099800420958068

Alkhateeb, A., Fain, P. R., Thody, A., Bennett, D. C., and Spritz, R. A. (2003). Epidemiology of vitiligo and associated autoimmune diseases in caucasian probands and their families. *Pigment Cell Res.* 16, 208–214. doi: 10.1034/j.1600-0749.2003.00032.x

Antrobus, M., Cuttell, S., and Machado, L. R. (2021). "Chapter 8 - Epigenetics, exercise, and the immune system," in *Epigenetics of Exercise and Sports*, ed. S. M. Raleigh (Cambridge, MA: Academic Press), 183–199.

Asea, A. (2007). Mechanisms of HSP72 release. *J. Biosci.* 32, 579–584. doi: 10.1007/s12038-007-0057-5

Ataş, H., and Gönül, M. (2017). Increased risk of metabolic syndrome in patients with vitiligo. *Balkan Med. J.* 34, 219–225. doi: 10.4274/balkanmedj.2016.1005

Azzazi, Y., Mostafa, W. Z., Sayed, K. S., Alhelf, M., Safwat, M., Mahrous, A., et al. (2021). Support for increased cardiovascular risk in non-segmental vitiligo among Egyptians: a hospital-based, case–control study. *Pigment Cell Melanoma Res.* 34, 598–604. doi: 10.1111/pcmr.12941

Barygina, V., Becatti, M., Lotti, T., Moretti, S., Taddei, N., and Fiorillo, C. (2015). Treatment with low-dose cytokines reduces oxidative-mediated injury in perilesional keratinocytes from vitiligo skin. *J. Dermatol. Sci.* 79, 163–170. doi: 10.1016/j.jdermsci.2015.05.003

Bengal, E., Aviram, S., and Hayek, T. (2020). p38 MAPK in glucose metabolism of skeletal muscle: beneficial or harmful? *Int. J. Mol. Sci.* 21:6480. doi: 10.3390/ijms21186480

Bergqvist, C., and Ezzedine, K. (2020). Vitiligo: a review. *Dermatology* 236, 571–592. doi: 10.1159/000506103

Bertolotti, A., Boniface, K., Vergier, B., Mossalayi, D., Taieb, A., Ezzedine, K., et al. (2014). Type I Interferon signature in the initiation of the immune response in vitiligo. *Pigment Cell Melanoma Res.* 27, 398–407. doi: 10.1111/pcmr.12219

Bhardwaj, S., Rani, S., Kumaran, M. S., Bhatia, A., and Parsad, D. (2020). Expression of Th17- and Treg-specific transcription factors in vitiligo patients. *Int. J. Dermatol.* 59, 474–481. doi: 10.1111/ijd.14766

Bishop, D. J., Granata, C., and Eynon, N. (2014). Can we optimise the exercise training prescription to maximise improvements in mitochondria function and content? *Biochim. Biophys. Acta* 1840, 1266–1275. doi: 10.1016/j.bbagen.2013.10.012

Bonomini, F., Rodella, L. F., and Rezzani, R. (2015). Metabolic syndrome, aging and involvement of oxidative stress. *Aging Dis.* 6, 109–120. doi: 10.14336/AD.2014.0305

Bonotis, K., Pantelis, K., Karaoulanis, S., Katsimaglis, C., Papaliaga, M., Zafiriou, E., et al. (2016). Investigation of factors associated with health-related quality of life and psychological distress in vitiligo. *J. Dtsch. Dermatol. Ges.* 14, 45–49. doi: 10.1111/ddg.12729

Carlos Henríquez-Olguín, S. B., Cabello-Verrugio, C, Jaimovich, E, Hidalgo, E, and Jensen, T. E. (2019). The emerging roles of nicotinamide adenine dinucleotide phosphate oxidase 2 in skeletal muscle redox signaling and metabolism. *Antioxid. Redox Signal.* 31, 1371–1410. doi: 10.1089/ars.2018.7678

D'Arino, A., Picardo, M., Truglio, M., Pacifico, A., and Iacovelli, P. (2021). Metabolic comorbidities in vitiligo: a brief review and report of new data from a single-center experience. *Int. J. Mol. Sci.* 22:8820. doi: 10.3390/ijms22168820

Das, S., Morvan, F., Jourde, B., Meier, V., Kahle, P., Brebbia, P., et al. (2015). ATP citrate lyase improves mitochondrial function in skeletal muscle. *Cell Metab.* 21, 868–876. doi: 10.1016/j.cmet.2015.05.006

de Sousa, C. V., Sales, M. M., Rosa, T. S., Lewis, J. E., de Andrade, R. V., and Simoes, H. G. (2016). The antioxidant effect of exercise: a systematic review and meta-analysis. *Sports Med.* 47, 277–293. doi: 10.1007/s40279-016-0566-1

Dell'Anna, M. L., Ottaviani, M., Albanesi, V., Vidolin, A. P., Leone, G., Ferraro, C., et al. (2007). Membrane lipid alterations as a possible basis for melanocyte degeneration in vitiligo. *J. Investig. Dermatol.* 127, 1226–1233. doi: 10.1038/sj.jid.5700700

Dell'Anna, M. L., Ottaviani, M., Bellei, B., Albanesi, V., Cossarizza, A., Rossi, L., et al. (2010). Membrane lipid defects are responsible for the generation of reactive oxygen species in peripheral blood mononuclear cells from vitiligo patients. *J. Cell Physiol.* 223, 187–193. doi: 10.1002/jcp.22027

Dell'Anna, M. L., Ottaviani, M., Kovacs, D., Mirabilii, S., Brown, D. A., Cota, C., et al. (2017). Energetic mitochondrial failing in vitiligo and possible rescue by cardiolipin. *Sci. Rep.* 7:13663. doi: 10.1038/s41598-017-13961-5

Dell'Anna, M. L., Urbanelli, S., Mastrofrancesco, A., Camera, E., Iacovelli, P., Leone, G., et al. (2003). Alterations of mitochondria in peripheral blood mononuclear cells of vitiligo patients. *Pigment Cell Melanoma Res.* 16, 553–559. doi: 10.1034/j.1600-0749.2003.00087.x

Demirbaş, A., Elmas, ÖF., Atasoy, M., Türsen, Ü, and Lotti, T. (2021). Can monocyte to HDL cholesterol ratio and monocyte to lymphocyte ratio be markers for inflammation and oxidative stress in patients with vitiligo? A preliminary study. *Arch. Dermatol. Res.* 313, 491–498. doi: 10.1007/s00403-020-02129-3

Deng, R., Zhang, P., Liu, W., Zeng, X., Ma, X., Shi, L., et al. (2018). HDAC is indispensable for IFN-γ-induced B7-H1 expression in gastric cancer. *Clin. Epigenet.* 10:153. doi: 10.1186/s13148-018-0589-6

Dimauro, I., Paronetto, M. P., and Caporossi, D. (2020). Exercise, redox homeostasis and the epigenetic landscape. *Redox Biol.* 35:101477. doi: 10.1016/j.redox.2020.101477

Ding, G.-Z., Zhao, W.-E., Li, X., Gong, Q.-L., and Lu, Y. (2015). A comparative study of mitochondrial ultrastructure in melanocytes from perilesional vitiligo skin and perilesional halo nevi skin. *Arch. Dermatol. Res.* 307, 281–289. doi: 10.1007/s00403-015-1544-4

Dwivedi, M., Laddha, N. C., Shah, K., Shah, B. J., and Begum, R. (2013). Involvement of interferon-gamma genetic variants and intercellular adhesion molecule-1 in onset and progression of generalized vitiligo. *J. Interferon Cytokine Res.* 33, 646–659. doi: 10.1089/jir.2012.0171

Edwardson, C. L., Gorely, T., Davies, M. J., Gray, L. J., Khunti, K., Wilmot, E. G., et al. (2012). Association of sedentary behaviour with metabolic syndrome: a meta-analysis. *PLoS One* 7:e34916. doi: 10.1371/journal.pone.0034916

Ellingsgaard, H., Hojman, P., and Pedersen, B. K. (2019). Exercise and health — emerging roles of IL-6. *Curr. Opin. Physiol.* 10, 49–54. doi: 10.1016/j.cophys.2019.03.009

Farrell, S. W., Finley, C. E., and Grundy, S. M. (2012). Cardiorespiratory fitness, LDL cholesterol, and CHD mortality in men. *Med. Sci. Sports Exerc.* 44, 2132–2137. doi: 10.1249/MSS.0b013e31826524be

Gafter, U., Malachi, T., Ori, Y., and Breitbart, H. (1997). The role of calcium in human lymphocyte DNA repair ability. *J. Lab. Clin. Med.* 130, 33–41. doi: 10.1016/S0022-2143(97)90056-1

Gifford, J. R., Garten, R. S., Nelson, A. D., Trinity, J. D., Layec, G., Witman, M. A., et al. (2016). Symmorphosis and skeletal muscle V̇O2 max: in vivo and in vitro measures reveal differing constraints in the exercise-trained and untrained human. *J. Physiol.* 594, 1741–1751. doi: 10.1113/JP271229

Giri, P. S., Dwivedi, M., Laddha, N. C., Begum, R., and Bharti, A. H. (2020). Altered expression of nuclear factor of activated T cells, forkhead box P3, and immune-suppressive genes in regulatory T cells of generalized vitiligo patients. *Pigment Cell Melanoma Res.* 33, 566–578. doi: 10.1111/pcmr.12862

Gjevestad, G. O., Holven, K. B., and Ulven, S. M. (2015). Effects of exercise on gene expression of inflammatory markers in human peripheral blood cells: a systematic review. *Curr. Cardiovasc. Risk Rep.* 9, 1–17. doi: 10.1007/s12170-015-0463-4

Glassman, S. J. (2014). "ROS and vitiligo," in *Systems Biology of Free Radicals and Antioxidants*, ed. I. Laher (Berlin: Springer), 3677–3695.

Gounder, S. S., Kannan, S., Devadoss, D., Miller, C. J., Whitehead, K. S., Odelberg, S. J., et al. (2012). Impaired transcriptional activity of Nrf2 in age-related myocardial oxidative stress is reversible by moderate exercise training. *PLoS One* 7:e45697. doi: 10.1371/journal.pone.0045697

Han, X., Yang, J., Yang, K., Zhao, Z., Abendschein, D. R., and Gross, R. W. (2007). Alterations in myocardial cardiolipin content and composition occur at the very earliest stages of diabetes: a shotgun lipidomics study. *Biochemistry* 46, 6417–6428. doi: 10.1021/bi7004015

Hasan, R., Agarwal, K., Podder, I., Misitzis, A., Schwartz, R. A., Wollina, U., et al. (2021). Simvastatin in vitiligo: an update with recent review of the literature. *Int. J. Dermatol.* 60, e390–e396. doi: 10.1111/ijd.15330

Hauff, K. D., Choi, S.-Y., Frohman, M. A., and Hatch, G. M. (2009). Cardiolipin synthesis is required to support human cholesterol biosynthesis from palmitate upon serum removal in Hela cells. *Can. J. Physiol. Pharmacol.* 87, 813–820. doi: 10.1139/Y09-055

Henríquez-Olguín, C., Díaz-Vegas, A., Utreras-Mendoza, Y., Campos, C., Arias-Calderón, M., Llanos, P., et al. (2016). NOX2 inhibition impairs early muscle gene expression induced by a single exercise bout. *Front. Physiol.* 7:282. doi: 10.3389/fphys.2016.00282

Hood, D. A., Tryon, L. D., Vainshtein, A., Memme, J., Chen, C., Pauly, M., et al. (2015). Exercise and the regulation of mitochondrial turnover. *Prog. Mol. Biol. Transl. Sci.* 135, 99–127. doi: 10.1016/bs.pmbts.2015.07.007

Horsburgh, S., Todryk, S., Toms, C., Moran, C. N., and Ansley, L. (2015). Exercise-conditioned plasma attenuates nuclear concentrations of DNA methyltransferase 3B in human peripheral blood mononuclear cells. *Physiol. Rep.* 3:e12621. doi: 10.14814/phy2.12621

Hou, X., Shi, J., Sun, L., Song, L., Zhao, W., Xiong, X., et al. (2022). The involvement of ERK1/2 and p38 MAPK in the premature senescence of melanocytes induced by H2O2 through a p53-independent p21 pathway. *J. Dermatol. Sci.* Online ahead of print., doi: 10.1016/j.jdermsci.2022.01.002

Jenkins, R., and Goldfarb, A. (1993). Introduction: oxidant stress, aging, and exercise. *Med. Sci. Sports Exerc.* 25, 210–212.

Jian, Z., Li, K., Song, P., Zhu, G., Zhu, L., Cui, T., et al. (2014). Impaired activation of the Nrf2-are signaling pathway undermines H2O2-induced oxidative stress response: a possible mechanism for melanocyte degeneration in vitiligo. *J. Investig. Dermatol.* 134, 2221–2230. doi: 10.1038/jid.2014.152

Jin, C.-H., Paik, I.-Y., Kwak, Y.-S., Jee, Y.-S., and Kim, J.-Y. (2015). Exhaustive submaximal endurance and resistance exercises induce temporary immunosuppression via physical and oxidative stress. *J. Exerc. Rehabil.* 11:198. doi: 10.12965/jer.150221

Jin, Y., Mailloux, C. M., Gowan, K., Riccardi, S. L., LaBerge, G., Bennett, D. C., et al. (2007). NALP1 in vitiligo-associated multiple autoimmune disease. *New England J. Med.* 356, 1216–1225. doi: 10.1056/NEJMoa061592

Karadag, A. S., Tutal, E., and Ertugrul, D. T. (2011). Insulin resistance is increased in patients with vitiligo. *Acta Dermato Venereologica* 91, 541–544. doi: 10.2340/00015555-1141

Kocer, B., Nazliel, B., Oztas, M., and Batur, H. (2009). Vitiligo and multiple sclerosis in a patient treated with interferon beta-1a: a case report. *Eur. J. Neurol.* 16, e78–e79. doi: 10.1111/j.1468-1331.2009.02563.x

Krüger, C., and Schallreuter, K. U. (2012). A review of the worldwide prevalence of vitiligo in children/adolescents and adults. *Int. J. Dermatol.* 51, 1206–1212. doi: 10.1111/j.1365-4632.2011.05377.x

Krüger, C., and Schallreuter, K. U. (2013). Cumulative life course impairment in vitiligo. *Curr. Probl. Dermatol.* 44, 102–117. doi: 10.1159/000350010

Krüger, K., and Mooren, F. C. (2014). Exercise-induced leukocyte apoptosis. *Exerc. Immunol. Rev.* 20, 117–134.

Laddha, N. C., Dwivedi, M., Gani, A. R., Shajil, E., and Begum, R. (2013). Involvement of superoxide dismutase isoenzymes and their genetic variants in progression of and higher susceptibility to vitiligo. *Free Radic. Biol. Med.* 65, 1110–1125. doi: 10.1016/j.freeradbiomed.2013.08.189

Laddha, N. C., Dwivedi, M., Mansuri, M. S., Singh, M., Gani, A. R., Yeola, A. P., et al. (2014). Role of oxidative stress and autoimmunity in onset and progression of vitiligo. *Exp. Dermatol.* 23, 352–353. doi: 10.1111/exd.12372

Le Poole, I. C., and Mehrotra, S. (2017). Replenishing regulatory T cells to halt depigmentation in vitiligo. *J. Investig. Dermatol. Symp. Proc.* 18, S38–S45. doi: 10.1016/j.jisp.2016.10.023

Lee, A.-Y., Youm, Y.-H., Kim, N.-H., Yang, H., and Choi, W.-I. (2004). Keratinocytes in the depigmented epidermis of vitiligo are more vulnerable to trauma (suction) than keratinocytes in the normally pigmented epidermis, resulting in their apoptosis. *Br. J. Dermatol.* 151, 995–1003. doi: 10.1111/j.1365-2133.2004.06136.x

Lee, E., Muñoz, C., McDermott, B., Beasley, K., Yamamoto, L., Hom, L., et al. (2015). Extracellular and cellular Hsp72 differ as biomarkers in acute exercise/environmental stress and recovery. *Scand. J. Med. Sci. Sports* 27, 66–74. doi: 10.1111/sms.12621

Leeuwenburgh, C., and Heinecke, J. (2001). Oxidative stress and antioxidants in exercise. *Curr. Med. Chem.* 8, 829–838. doi: 10.2174/0929867013372896

Lionaki, E., Markaki, M., Palikaras, K., and Tavernarakis, N. (2015). Mitochondria, autophagy and age-associated neurodegenerative diseases: new insights into a complex interplay. *Biochim. Biophys. Acta* 1847, 1412–1423. doi: 10.1016/j.bbabio.2015.04.010

Lira, F. S., Rosa, J. C., Lima-Silva, A. E., Souza, H. A., Caperuto, E. C., Seelaender, M. C., et al. (2010). Sedentary subjects have higher PAI-1 and lipoproteins levels than highly trained athletes. *Diabetol. Metab. Syndr.* 2:7. doi: 10.1186/1758-5996-2-7

Little, J. P., Gillen, J. B., Percival, M. E., Safdar, A., Tarnopolsky, M. A., Punthakee, Z., et al. (2011). Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *J. Appl. Physiol.* 111, 1554–1560. doi: 10.1152/japplphysiol.00921.2011

Lotti, T., and D'Erme, A. M. (2014). Vitiligo as a systemic disease. *Clin. Dermatol.* 32, 430–434.

Lotti, T., Hercogova, J., and Fabrizi, G. (2015). Advances in the treatment options for vitiligo: activated low-dose cytokines-based therapy. *Exp. Opin. Pharmacother.* 16, 2485–2496. doi: 10.1517/14656566.2015.1087508

Lu, J., and Holmgren, A. (2014). The thioredoxin antioxidant system. *Free Radic. Biol. Med.* 66, 75–87.

Lu, Z., Xu, Y., Song, Y., Bíró, I., and Gu, Y. (2021). A mixed comparisons of different intensities and types of physical exercise in patients with diseases related to oxidative stress: a systematic review and network meta-analysis. *Front. Physiol.* 12:700055. doi: 10.3389/fphys.2021.700055

Ma, S.-H., Ang, M.-D., Chang, Y.-T., and Dai, Y.-X. (2021). Association between vitiligo and hearing loss: a systemic review and metaanalysis. *J. Am. Acad. Dermatol.* 85, 1465–1472. doi: 10.1016/j.jaad.2020.12.029

Maresca, V., Roccella, M., Roccella, F., Camera, E., Del Porto, G., Passi, S., et al. (1997). Increased sensitivity to peroxidative agents as a possible pathogenic factor of melanocyte damage in vitiligo. *J. Investig. Dermatol.* 109, 310–313. doi: 10.1111/1523-1747.ep12335801

Margaritelis, N. V., Theodorou, A. A., Paschalis, V., Veskoukis, A. S., Dipla, K., Zafeiridis, A., et al. (2018). Adaptations to endurance training depend on exercise-induced oxidative stress: exploiting redox interindividual variability. *Acta Physiol.* 222:12898. doi: 10.1111/apha.12898

Martins, C., Migayron, L., Drullion, C., Jacquemin, C., Lucchese, F., Rambert, J., et al. (2021). Vitiligo skin T cells are prone to produce type 1 and type 2 cytokines to induce melanocyte dysfunction and epidermal inflammatory response through jak signaling. *J. Invest. Dermatol.* Online ahead of print., doi: 10.1016/j.jid.2021.09.015,

Minuzzi, L. G., Rama, L., Bishop, N. C., Rosado, F., Martinho, A., Paiva, A., et al. (2017). Lifelong training improves anti-inflammatory environment and maintains the number of regulatory T cells in masters athletes. *Eur. J. Appl. Physiol.* 117, 1131–1140. doi: 10.1007/s00421-017-3600-6

Minuzzi, L. G., Rama, L., Chupel, M. U., Rosado, F., Dos Santos, J. V., Simpson, R., et al. (2018). Effects of lifelong training on senescence and mobilization of T lymphocytes in response to acute exercise. *Exerc. Immunol. Rev.* 24, 72–84.

Monteiro, P. A., Campos, E. Z., de Oliveira, F. P., Peres, F. P., Rosa-Neto, J. C., Pimentel, G. D., et al. (2017). Modulation of inflammatory response arising from high-intensity intermittent and concurrent strength training in physically active males. *Cytokine* 91, 104–109. doi: 10.1016/j.cyto.2016.12.007

Mosenson, J. A. (2013). *Defining a Role for Inducible Heat Shock Protein 70 (Hsp70i) in Mediating Autoimmune Vitiligo*. Ph.D. thesis, Doctoral dissertation, Loyola University Chicago, Chicago.

Muthusamy, V. R., Kannan, S., Sadhaasivam, K., Gounder, S. S., Davidson, C. J., Boeheme, C., et al. (2012). Acute exercise stress activates Nrf2/ARE signaling and promotes antioxidant mechanisms in the myocardium. *Free Radic. Biol. Med.* 52, 366–376. doi: 10.1016/j.freeradbiomed.2011.10.440

Namazi, N., Amani, M., Haghighatkhah, H. R., Noori, E., and Abdollahimajd, F. (2021). Increased risk of subclinical atherosclerosis and metabolic syndrome in patients with vitiligo: a real association or a coincidence? *Dermatol. Ther.* 34:e14803. doi: 10.1111/dth.14803

Natarajan, V. T., Ganju, P., Singh, A., Vijayan, V., Kirty, K., Yadav, S., et al. (2014). IFN-γ signaling maintains skin pigmentation homeostasis through regulation of melanosome maturation. *Proc. Natl. Acad. Sci.* 111, 2301–2306. doi: 10.1073/pnas.1304988111

Ni, J., Qiu, L.-J., Zhang, M., Wen, P.-F., Ye, X.-R., Liang, Y., et al. (2014). CTLA-4 CT60 (rs3087243) polymorphism and autoimmune thyroid diseases susceptibility: a comprehensive meta-analysis. *Endocr. Res.* 39, 180–188. doi: 10.3109/07435800.2013.879167

Nilsson, A., Björnson, E., Flockhart, M., Larsen, F. J., and Nielsen, J. (2019). Complex I is bypassed during high intensity exercise. *Nat. Commun.* 10, 1–11. doi: 10.1038/s41467-019-12934-8

Ortona, E., Maselli, A., Delunardo, F., Colasanti, T., Giovannetti, A., and Pierdominici, M. (2014). Relationship between redox status and cell fate in immunity and autoimmunity. *Antioxid. Redox Signal.* 21, 103–122. doi: 10.1089/ars.2013.5752

Pannala, V. R., and Dash, R. K. (2014). Mechanistic characterization of the thioredoxin system in the removal of hydrogen peroxide. *Free Radic. Biol. Med.* 78, 42–55. doi: 10.1016/j.freeradbiomed.2014.10.508

Pareja-Galeano, H., Sanchis-Gomar, F., and García-Giménez, J. L. (2014). Physical exercise and epigenetic modulation: elucidating intricate mechanisms. *Sports Med.* 44, 429–436. doi: 10.1007/s40279-013-0138-6

Périard, J. D., Travers, G. J. S., Racinais, S., and Sawka, M. N. (2016). Cardiovascular adaptations supporting human exercise-heat acclimation. *Auton. Neurosci.* 196, 52–62. doi: 10.1016/j.autneu.2016.02.002

Qi, J., Luo, X., Ma, Z., Zhang, B., Li, S., Duan, X., et al. (2020). Swimming exercise protects against insulin resistance via regulating oxidative stress through nox4 and akt signaling in high-fat diet-fed mice. *J. Diabetes Res.* 2020:2521590. doi: 10.1155/2020/2521590

Radak, Z., Hart, N., Marton, O., and Koltai, E. (2014). "Regular exercise results in systemic adaptation against oxidative stress," in *Systems Biology of Free Radicals and Antioxidants*, ed. I. Laher (Berlin: Springer), 3855–3869. doi: 10.1055/s-0029-1233464

Rashighi, M., Agarwal, P., Richmond, J. M., Harris, T. H., Dresser, K., Su, M.-W., et al. (2014). CXCL10 is critical for the progression and maintenance of depigmentation in a mouse model of vitiligo. *Sci. Transl. Med.* 6:223ra223.

Rhind, S. G., Shek, P. N., Shinkai, S., and Shephard, R. J. (1996). Effects of moderate endurance exercise and training on in vitro lymphocyte proliferation, interleukin-2 (IL-2) production, and IL-2 receptor expression. *Eur. J. Appl. Physiol. Occup. Physiol.* 74, 348–360. doi: 10.1007/bf02226932

Riding, R. L., and Harris, J. E. (2019). The role of memory CD8+ T cells in vitiligo. *J. Immunol.* 203, 11–19. doi: 10.4049/jimmunol.1900027

Sansbury, B. E., Jones, S. P., Riggs, D. W., Darley-Usmar, V. M., and Hill, B. G. (2011). Bioenergetic function in cardiovascular cells: the importance of the reserve capacity and its biological regulation. *Chem. Biol. Interactions* 191, 288–295. doi: 10.1016/j.cbi.2010.12.002

Schallreuter, K., Pittelkow, M., and Wood, J. (1991a). Defects in antioxidant defense and calcium transport in the epidermis of xeroderma pigmentosum patients. *Arch. Dermatol. Res.* 283, 449–455. doi: 10.1007/BF00371781

Schallreuter, K. U., Wood, J. M., and Berger, J. (1991b). Low catalase levels in the epidermis of patients with vitiligo. *J. Investig. Dermatol.* 97, 1081–1085. doi: 10.1111/1523-1747.ep12492612

Schallreuter, K. U. (2014). "Reactive oxygen species and reactive nitrogen species in vitiligo," in *Systems Biology of Free Radicals and Antioxidants*, ed. I. Laher (Berlin: Springer), 3697–3736.

Schallreuter, K. U., Salem, M. A. E. L., Gibbons, N. C. J., Martinez, A., Slominski, R., Lüdemann, J., et al. (2012). Blunted epidermal l-tryptophan metabolism in vitiligo affects immune response and ROS scavenging by Fenton chemistry, part 1: epidermal H2O2/ONOO–mediated stress abrogates tryptophan hydroxylase and dopa decarboxylase activities, leading to low serotonin and melatonin levels. *FASEB J.* 26, 2457–2470. doi: 10.1096/fj.11-197137

Schallreuter, K. U., and Wood, J. M. (1991). Sensitivity and resistance in human metastatic melanoma to the new chloroethylnitrosourea anti-tumor drug fotemustine. *Biochim. Biophys. Acta* 1096, 277–283. doi: 10.1016/0925-4439(91)90063-f

Schallreuter-Wood, K. U., Pittelkow, M. R., and Swanson, N. N. (1996). Defective calcium transport in vitiliginous melanocytes. *Arch. Dermatol. Res.* 288, 11–13. doi: 10.1007/BF02505036

Schlame, M., and Greenberg, M. L. (2017). Biosynthesis, remodeling and turnover of mitochondrial cardiolipin. *Biochim. Biophys. Acta* 1862, 3–7. doi: 10.1016/j.bbalip.2016.08.010

Shajil, E., and Begum, R. (2006). Antioxidant status of segmental and non-segmental vitiligo. *Pigment Cell Res.* 19, 179–180. doi: 10.1111/j.1600-0749.2006.00299.x

Sharma, L. K., Lu, J., and Bai, Y. (2009). Mitochondrial respiratory complex I: structure, function and implication in human diseases. *Curr. Med. Chem.* 16, 1266–1277. doi: 10.2174/092986709787846578

Sharma, Y. K., Bansal, P., Menon, S., and Prakash, N. (2017). Metabolic syndrome in vitiligo patients among a semi-urban Maharashtrian population: a case control study. *Diabetes Metab. Syndr.* 11, S77–S80. doi: 10.1016/j.dsx.2016.12.009

Shaw, D. M., Merien, F., Braakhuis, A., and Dulson, D. (2017). T-cells and their cytokine production: the anti-inflammatory and immunosuppressive effects of strenuous exercise. *Cytokine* 104, 136–142. doi: 10.1016/j.cyto.2017.10.001

Shi, Q., Zhang, W., Guo, S., Jian, Z., Li, S., Li, K., et al. (2016). Oxidative stress-induced overexpression of miR-25: the mechanism underlying the degeneration of melanocytes in vitiligo. *Cell Death Differentiation* 23, 496–508. doi: 10.1038/cdd.2015.117

Shi, Y.-L., Weiland, M., Li, J., Hamzavi, I., Henderson, M., Huggins, R. H., et al. (2013). MicroRNA expression profiling identifies potential serum biomarkers for non-segmental vitiligo. *Pigment Cell Melanoma Res.* 26, 418–421. doi: 10.1111/pcmr.12086

Shiu, M. (2016). *Modulation of T Cell Distribution and Function by High-Intensity Interval Training*. Toronto, ON: University of Toronto.

Skurkovich, B., and Skurkovich, S. (2006). "Inhibition of IFN-γ as a method of treatment of various autoimmune diseases, including skin diseases," in *Cytokines as Potential Therapeutic Targets for Inflammatory Skin Diseases*, eds R. Numerof, C. A. Dinarello, and K. Asadullah (Berlin: Springer), 1–27. doi: 10.1007/3-540-37673-9_1

Song, G. G., Kim, J.-H., and Lee, Y. H. (2013). The CTLA-4 +49A/G, CT60A/G and PTPN22 1858 C/T polymorphisms and susceptibility to vitiligo: a meta-analysis. *Mol. Biol. Rep.* 40, 2985–2993. doi: 10.1007/s11033-012-2370-9

Stramucci, L., Pranteda, A., and Bossi, G. (2018). Insights of crosstalk between p53 protein and the mkk3/mkk6/p38 mapk signaling pathway in cancer. *Cancers* 10:131. doi: 10.3390/cancers10050131

Tanacan, E., and Atakan, N. (2020). Higher incidence of metabolic syndrome components in vitiligo patients: a prospective cross-sectional study☆,☆☆. *An. Bras. Dermatol.* 95, 165–172. doi: 10.1016/j.abd.2019.07.006

Tarpey, M. D., Davy, K. P., McMillan, R. P., Bowser, S. M., Halliday, T. M., Boutagy, N. E., et al. (2017). Skeletal muscle autophagy and mitophagy in endurance-trained runners before and after a high-fat meal. *Mol. Metab.* 6, 1597–1609. doi: 10.1016/j.molmet.2017.10.006

Teulings, H., Overkamp, M., Ceylan, E., Nieuweboer-Krobotova, L., Bos, J., Nijsten, T., et al. (2013). Decreased risk of melanoma and nonmelanoma skin cancer in patients with vitiligo: a survey among 1307 patients and their partners. *Br. J. Dermatol.* 168, 162–171. doi: 10.1111/bjd.12111

Tsai, M.-F., Jiang, D., Zhao, L., Clapham, D., and Miller, C. (2014). Functional reconstitution of the mitochondrial Ca2+/H+ antiporter Letm1. *J. Gen. Physiol.* 143, 67–73. doi: 10.1085/jgp.201311096

Turner, J. E., Wadley, A. J., Aldred, S., Fisher, J. P., Bosch, J. A., and Campbell, J. P. (2016). Intensive exercise does not preferentially mobilize skin-homing T cells and NK cells. *Med. Sci. Sports Exerc.* 48, 1285–1293. doi: 10.1249/MSS.0000000000000914

Vainshtein, A., Tryon, L. D., Pauly, M., and Hood, D. A. (2015). Role of PGC-1α during acute exercise-induced autophagy and mitophagy in skeletal muscle. *Am. J. Physiol. Cell Physiol.* 308, C710–C719. doi: 10.1152/ajpcell.00380.2014

Vargas-Mendoza, N., Morales-González, Á, Madrigal-Santillán, E. O., Madrigal-Bujaidar, E., Álvarez-González, I., García-Melo, L. F., et al. (2019). Antioxidant and adaptative response mediated by nrf2 during physical exercise. *Antioxidants* 8:196. doi: 10.3390/antiox8060196

Venditti, P., Masullo, P., and Di Meo, S. (1999). Effect of training on H2 O2 release by mitochondria from rat skeletal muscle. *Arch. Biochem. Biophys.* 372, 315–320.

Verma, D., Hussain, K., Namiq, K. S., Firoz, A., Bouchama, M., Raza, M., et al. (2021). Vitiligo: the association with metabolic syndrome and the role of simvastatin as an immunomodulator. *Cureus* 13:e14029. doi: 10.7759/cureus.14029

Wang, Y., Wang, K., Liang, J., Yang, H., Dang, N., Yang, X., et al. (2015). Differential expression analysis of miRNA in peripheral blood mononuclear cells of patients with non-segmental vitiligo. *J. Dermatol.* 42, 193–197. doi: 10.1111/1346-8138.12725

Xie, H., Zhou, F., Liu, L., Zhu, G., Li, Q., Li, C., et al. (2016). Vitiligo: how do oxidative stress-induced autoantigens trigger autoimmunity? *J. Dermatol. Sci.* 81, 3–9. doi: 10.1016/j.jdermsci.2015.09.003

You, S., Cho, Y. H., Byun, J. S., and Shin, E. C. (2013). Melanocyte-specific CD8+ T cells are associated with epidermal depigmentation in a novel mouse model of vitiligo. *Clin. Exp. Immunol.* 174, 38–44. doi: 10.1111/cei.12146

Zhang, Y., Liu, L., Jin, L., Yi, X., Dang, E., Yang, Y., et al. (2014). Oxidative stress–induced calreticulin expression and translocation: new insights into the destruction of melanocytes. *J. Investig. Dermatol.* 134, 183–191. doi: 10.1038/jid.2013.268

Zhang, Z., Yang, X., Liu, O., Cao, X., Tong, J., Xie, T., et al. (2021). Differentially expressed microRNAs in peripheral blood mononuclear cells of non-segmental vitiligo and their clinical significance. *J. Clin. Lab. Anal.* 35:e23648. doi: 10.1002/jcla.23648

Zhao, M., Gao, F., Wu, X., Tang, J., and Lu, Q. (2010). Abnormal DNA methylation in peripheral blood mononuclear cells from patients with vitiligo. *Br. J. Dermatol.* 163, 736–742. doi: 10.1111/j.1365-2133.2010.09919.x

Zhou, L., Lim, H. W., and Mi, Q.-S. (2019). "Epigenetics," in *Vitiligo*, eds M. Picardo and A. Taïeb (Cham: Springer International Publishing), 253–264.

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frontiers in Physiology

ORIGINAL RESEARCH
published: 24 March 2022
doi: 10.3389/fphys.2022.836484

Dietary Intervention Associated With Moderate-Intensity Continuous Training Leads to Changes in the Inflammatory Profile in Visceral Adipose Tissue but Not in Skeletal Muscle in Diet-Induced Obese Rats

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OPEN ACCESS

Edited by:
Giuseppe D'Antona, University of Pavia, Italy

Reviewed by:
Patricia Chimin, State University of Londrina, Brazil
Santosh Kumar Maurya, The Ohio State University, United States

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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *15 December 2021*
Accepted: *10 February 2022*
Published: *24 March 2022*

Citation:
Costa JLF, Furino VO, Castro CA, Alves JM and Duarte ACGO (2022) Dietary Intervention Associated With Moderate-Intensity Continuous Training Leads to Changes in the Inflammatory Profile in Visceral Adipose Tissue but Not in Skeletal Muscle in Diet-Induced Obese Rats. Front. Physiol. 13:836484. doi: 10.3389/fphys.2022.836484

This study aimed to determine the concentrations of inflammatory markers in visceral adipose tissue (VAT) and skeletal muscle, and changes in body mass and adipocyte size in diet-induced obese rats after moderate-intensity continuous training (MICT) and/or dietary intervention. After 8 weeks of obesity induction through a high-fat diet (HFD) consumption, twenty diet-induced obese male Wistar rats were divided into four groups as follows: (i) control rats fed with HFD (HFD-SED), (ii) obese rats fed with HFD and submitted to MICT (HFD-MICT), (iii) obese rats that were submitted to a nutritional intervention by switching HFD to chow diet (CD-SED), and (iv) obese rats that were submitted to MICT and nutritional intervention (CD-MICT). All the animals in the training groups were submitted to MICT, with an intensity of 50–85% of V_{max}, 60 min/day, 3 days/week for 8 weeks. Gastrocnemius muscle (GAST) and mesenteric adipose tissue (mWAT) were collected to quantify tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, and IL-10 using ELISA. The body mass was recorded before and after the experimental protocols, and the adipocyte morphology was assessed using histological analysis. The results showed that HFD-SED had higher body mass, higher concentrations of inflammatory markers in mWAT, and higher increase in adipocyte size. The CD-SED and CD-MICT groups presented with reduced body mass, relative weight of mWAT, and adipocyte size. Moreover, the inflammatory markers in mWAT were reduced after dietary intervention (TNF-α), MICT (IL-10 and TNF-α), or both interventions combined (IL-6 and

TNF-α). In contrast, there was no reduction in GAST-relative weight or concentrations of inflammatory markers for any treatment. Finally, we concluded that 8 weeks of dietary intervention alone and combined with MICT were effective in reducing some of the deleterious effects caused by obesity.

Keywords: inflammatory markers, visceral adipose tissue, skeletal muscle, moderate-intensity continuous training, obesity

INTRODUCTION

Changes in lifestyle, including a sedentary condition and consumption of an obesogenic diet (high fat and sugar), have contributed to the high prevalence of obesity and type 2 diabetes worldwide (Bray et al., 2016; Gopalan et al., 2021). The sedentary lifestyle associated with consuming a high-fat diet (HFD) induces a dynamic reprogramming in cellular metabolism, which affects insulin signaling and oxidative phosphorylation, and leads to metabolic syndrome (Chouchani and Kajimura, 2019). In addition, adipose tissue is a key driver of metabolic disturbance induced by obesity (Chouchani and Kajimura, 2019).

Excessive accumulation of visceral adipose tissue (VAT), which wraps around the internal organs (mesenteric fat), serves as the main site of inflammatory response through immune-cell infiltration and the release of inflammatory adipokines during positive energy balance conditions (Cao et al., 2021). Chronic low-grade inflammation in VAT involves an unhealthy expansion of adipocyte size (hypertrophy) (Jais and Brüning, 2017), local hypoxia, adipocyte death, mechanical stress, which leads to an excessive release of fatty acids and pro-inflammatory adipokines, such as interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α), which are associated with metabolic disorders, including insulin resistance, hepatic steatosis, and cardiovascular diseases (Chouchani and Kajimura, 2019; Han et al., 2020; Kawai et al., 2021). Hence, adipose tissue serves as the hub of inflammation throughout the body (Zatterale et al., 2020). Thus, strategies that seek to recover from the inflammatory state of VAT are needed to restore cellular homeostasis and treat the obesity condition.

Aerobic exercise is an important non-pharmacological strategy to control obesity and can act as a preventative treatment against metabolic syndrome by stimulating visceral fat loss (Bellicha et al., 2021). The benefits of aerobic exercise on metabolic health include increased oxidative phosphorylation, improved insulin sensitivity, reduced release of pro-inflammatory adipokines, and the production of myokines that improve metabolic flexibility (Lancaster and Febbraio, 2014; Batatinha et al., 2019). In fact, it is well established that appropriate aerobic exercises stimulate the release of myokines with anti-inflammatory functions (Pedersen et al., 2007; Leal et al., 2018) and preserve the healthy function of adipose tissue (Lancaster and Febbraio, 2014). In addition, significant increases in the circulation of IL-6 during prolonged exercises were observed in previous studies (Steensberg et al., 2000, 2003), and further findings detected other myokines that are involved in this process, such as IL-8, IL-1 receptor antagonist (IL-1ra), IL-15, and irisin (Bay and Pedersen, 2020; Gonzalez-Gil and Elizondo-Montemayor, 2020).

One of the widely used physical exercise modalities aiming to reduce the deleterious effects of obesity is moderate-intensity continuous training (MICT), which is characterized by the intensities between 50–75% of maximum heart rate (HR_{max}) (Wewege et al., 2017), 65–70% of maximum speed (V_{max}) (Khalafi et al., 2020), and 65–70% of maximal oxygen consumption (VO_{2max}) (Wang et al., 2017). MICT has been shown to be effective in modulating the weight gain of obese animals (Wang et al., 2017), promoting improvements in maximum exercise capacity (MEC) (Khalafi et al., 2020), reducing adipocyte expansion (Lacerda et al., 2019; Khalafi et al., 2020), and producing anti-inflammatory effects in adipose tissue (Lira et al., 2012) and skeletal muscle (Gopalan et al., 2021).

Another non-pharmacological strategy used to control obesity is dietary intervention (Longo and Panda, 2016). These approaches can occur through changes in the time of food supply (Aouichat et al., 2020), reducing the amount of food offered (Lowe et al., 2020), or through modulation in the nutrient composition. Despite this, many of these studies carried out interventions that combined the induction of obesity by HFD concomitantly with the beginning of MICT training and/or performed dietary interventions based on calorie restrictions. However, a few of them sought to investigate the effects of MICT associated with a dietary intervention without diets based on caloric restriction after an obesity induction period (Furino et al., 2021).

In view of this, some remaining questions were as follows: "Is it necessary to have five MICT sessions per week?" "If we associate training in fewer weekly sessions with a dietary intervention, could it lead to the same adaptations?" "Would the magnitude be the same?" Thus, the aim of this study was to investigate the effects of three MICT weekly sessions, dietary intervention, or both interventions combined, on the modulation of the inflammatory profile (IL-6, TNF-α, and IL-10) of skeletal muscle and VAT in rats with diet-induced obesity. Therefore, we hypothesized that aerobic exercise and dietary intervention may attenuate the inflammatory response induced by obesity in adipose tissue and that the combination of training plus dietary intervention will be able to induce a better reduction in the inflammatory response as part of a regulatory loop that increases the release of myokines by skeletal muscle.

MATERIALS AND METHODS

Ethic and Experimental Groups

Twenty male *Rattus norvegicus albinus* (Wistar) rats with an initial weight of ≃300 g (45-day-old) were kept in

polypropylene cages (five animals per cage) with food and water provided *ad libitum*. The environmental conditions were controlled with temperature and humidity maintained at 22–24°C and 50–60%, respectively, and a photoperiod of 12 h light/dark cycle (lights on at 6 p.m.). The Ethics Committee on Animal Use at the Federal University of São Carlos (São Paulo, Brazil) approved all experimental procedures under protocol no. 7631210617.

Experimental Design

All animals were subjected to the same HFD for 8 weeks. After 8 weeks of diet-induced obesity, the rats were randomly divided into four groups: (i) control rats fed with HFD (HFD-SED), (ii) obese rats fed with HFD and submitted to MICT (HFD-MICT), (iii) obese rats that were submitted to nutritional intervention by switching HFD for chow diet (CD-SED), and (iv) obese rats that were submitted to MICT and nutritional intervention (CD-MICT). The sedentary groups (CD-SED and HFD-SED) were kept in their cages during the experimental period without any type of exercise, and the groups that underwent training (CD-MICT and HFD-MICT) performed 8 weeks of treadmill running.

Diets

The experimental groups received the standard rat chow diet Agromix® (Jaboticabal, SP, Brazil) in pellet form, which contained ∼23 g of protein, ∼39 g of carbohydrates, ∼4.8 g of total fat, and ∼5 g of fiber per 100 g of diet, or a palatable HFD that consisted of standard rat chow plus peanuts, milk chocolate, and sweet biscuits at a proportion of 3:2:2:1 that was mixed in pellet form (Estadella et al., 2004), containing ∼18 g of protein, ∼33 g of carbohydrate, ∼20 g of total fat, and ∼3 g of fiber per 100 g of diet (Costa et al., 2021).

Body Mass and Food Intake Measurement

The body mass was measured once a week, and food intake was calculated by the difference in weight between the amount of food offered and the amount of food remaining every 2–3 days.

Moderate-Intensity Continuous Training Protocol

Adaptation

Adaptation aimed to minimize the stress that can occur with chronic physical training without promoting physiological adaptations resulting from training (Kunstetter et al., 2018). Thus, before starting the training protocol, in the 8 week of obesity induction, the animals were familiarized with running on the treadmill adapted for rats for 5 consecutive days (10–15 min/day; 6–10 m/min) (Furino et al., 2021). Furthermore, to select the animals that would compose the training groups (MICT), an evaluation of physical performance was carried out (Rachetti et al., 2013). Following the evaluation model proposed by Rachetti et al. (2013), the animals selected for the MICT groups were those that had the highest arithmetic mean (on a scale of 1–5) during 5 days of adaptation.

Maximal Exercise Capacity

To determine the running speed used during the physical training protocol and evaluate the adaptations generated by treadmill training, the maximal exercise capacity (MEC) test was performed. The animals were placed on the treadmill and allowed to adapt over a 5-min period of 6 m/min without elevation, and the speed was gradually increased by 2 m/min every 2 min until they were unable to maintain the running pattern through increases in treadmill speed (Souza et al., 2018). The 100% of MEC is defined as the maximum speed (in m/min) and is used to determine the intensity of training sessions. Additionally, the MEC test was performed in the fourth week to adjust the training intensity and after 48 h in the last training session. Throughout the procedure, electric shocks were not used as a form of stimulation, but mechanical pressure was applied to the distal part of the tail.

Moderate-Intensity Continuous Training Protocol

The training protocol consisted of running sessions on a treadmill adapted for rats, containing six individual tracks separated by bays made of acrylic, always between 8 and 12 a.m. respecting the animals' light-dark cycle. The training protocol had a frequency of three weekly sessions, for 8 weeks, 60 min per session at an intensity of 50–80% of the maximum speed (V_{max}). Each training session was divided into three parts: 10 min for warmup (0–5 min: 50% of V_{max}; 5–10 min: 60% of V_{max}); 40 min for the main part (65–80% of V_{max}); and 10 min for cool down (50% of V_{max}) (Furino et al., 2021).

Experiment and Tissue Collection

Animals were euthanized by decapitation 48 h after the last treadmill running session. The mesenteric adipose tissue (mWAT) and gastrocnemius muscle (GAST) were immediately removed, weighed, and stored at −80°C for further analysis. Additionally, a sample of mWAT was excised and fixed in 10% formalin to perform histological analysis.

Histological Analysis

After being excised and fixed in 10% formalin, the mWAT samples were dehydrated in a grade alcohol series and the paraffin-embedded samples were cut (5 μm) using a microtome and mounted on glass slides that were then stained with H&E. The slides that were stained with H&E were digitized in a scanner (Pannoramic Digital Slide Scanners system, 3DHISTECH, Ltd., Hungary). In this study, we randomly digitized five images for each animal at 20× objective (Pannoramic Viewer), and then the Adiposoft (v. 1.15) plug-in of ImageJ Fiji (v 2.0.0) (National Institutes of Health, United States) was used to quantify the size (μm^2) of the adipocytes (Galarraga et al., 2012; Parlee et al., 2014).

TABLE 1 | Changes in body mass (g) and food intake.

Groups	Body mass (g)				Daily consumption (g/day)	Caloric intake (kcal/day)
	Week 0	Week 8	Week 16	Δ % (W^{16}–W^{8})		
HFD-SED	400.2 ± 12.3	559.6 ± 28.3	641.7 ± 33.9	14.6 ± 1.6	23.33 ± 0.7	108.9 ± 3.4
HFD-MICT	392.8 ± 7.0	551.4 ± 20.1	588.6 ± 17.3	6.9 ± 1.9	21.38 ± 0.5	99.8 ± 2.7
CD-SED	359.6 ± 23.6	465.0 ± 40.6	470.4 ± 32.7[a]	2.0 ± 3.2[a]	23.30 ± 1.0	89.9 ± 4.1[a]
CD-MICT	370.0 ± 21.1	488.0 ± 32.7	474.8 ± 34.9[a]	−2.6 ± 3.6[a]	25.00 ± 1.3	96.4 ± 5.0

Data presented as mean ± SEM ($p < 0.05$).
HFD-SED, high-fat diet sedentary (n = 5); HFD-MICT, high-fat diet plus MICT (n = 5); CD-SED, chow diet sedentary (n = 5); CD-MICT, chow diet plus MICT (n = 5).
[a] vs. HFD-SED.

TABLE 2 | Relative weight of depots (g/100 BM).

Relative weight of depots (g/100 of body mass)		
Groups	**mWAT**	**GASTROC**
HFD-SED	1.99 ± 0.32	0.43 ± 0.03
HFD-MICT	1.10 ± 0.18	0.47 ± 0.01
CD-SED	0.61 ± 0.09[a]	0.50 ± 0.00
CD-MICT	0.44 ± 0.05[a]	0.52 ± 0.01

Data presented as mean ± SEM ($p < 0.05$).
mWAT, mesenteric adipose tissue; GAST, gastrocnemius muscle; HFD-SED, high-fat diet sedentary (n = 5); HFD-MICT, high-fat diet plus MICT (n = 5); CD-SED, chow diet sedentary (n = 5); CD-MICT, chow diet plus MICT (n = 5).
[a] vs. HFD-SED.

TABLE 3 | Variables of exercise.

		HFD-MICT	CD-MICT
Pre-exercise	MEC (m/min)	26.80 ± 0.49[a]	21.20 ± 0.49
	Time to exhaustion (min)	21.20 ± 0.49[a]	16.00 ± 1.09
	Distance covered (m)	568.80 ± 21.48[a]	340.80 ± 29.56
Post-exercise	MEC (m/min)	33.60 ± 0.74[c]	34.40 ± 0.74[c]
	Time to exhaustion (min)	29.60 ± 0.74[c]	30.40 ± 0.67[c]
	Distance covered (m)	996.80 ± 47.68[c]	1047.60 ± 45.08[c]
Δ of MEC (%)		25.36 ± 1.80	62.72 ± 5.99[b]

Data presented as mean ± SEM ($p < 0.05$).
HFD-MICT, high-fat diet plus MICT (n = 5); CD-MICT, chow diet plus MICT (n = 5).
[a] vs. CD-MICT.
[b] vs. HFD-MICT.
[c] Pre-exercise vs. post-exercise in the same group.

Assessment of Mesenteric Adipose Tissue and Gastrocnemius Muscle Tissue Protein and Cytokine Concentrations

The samples of mWAT (∼200 mg) and GAST (∼100 mg) were homogenized in 600 and 500 μl, respectively, with an extraction buffer containing SDS 0.1% (p/v), Triton 1% (v/v), Tris–HCl pH 7.8, 50 mM, NaCl 150 mM, EDTA 15 mM, EGTA 5 mM, and protease inhibitors Complete Mini Roche® 1× (Sigma-Aldrich, St. Louis, MO, United States). The mWAT samples were homogenized by rapid shaking with ceramic beads (2 × 30 s at 6 m/s) and GAST (3 × 30 s at 4 m/s) using a high-speed benchtop homogenizer FastPrep-24™ (MP Biomedicals, CA, United States). Then, the homogenate was centrifuged for 10 min at 10,000 × *g* for mWAT, whereas GAST was centrifuged for 15 min at 10,621 × *g*, both at a temperature of 4°C (Eppendorf® Centrifuge 5430/5430R, Germany). The supernatant was transferred to a sterile microtube and brought to −80°C for further analysis.

The protein quantification of the supernatant was determined through the colorimetric assay of proteins based on bicinchoninic acid (BCA, AR0146-500) following the specifications of the BCA Protein Assay Kit (Boster Biological Technology, Pleasanton, CA, United States) and read in an automated plate reader (SpectraMax i3x machine, Molecular Devices, San Jose, CA, United States). According to the manufacturer's instructions, IL-6, TNF-α, and IL-10 concentrations measured in lysate tissues were determined by ELISA at a 1:2 dilution. The method followed the specifications of the corresponding BD Biosciences Pharmingen® (San Diego, CA, United States) kits (IL-6: Cat. No. 550319; IL-10: Cat. No. 555134; TNF-α: Cat. No. 558535). The concentrations of the samples were calculated from the concentration curve of the cytokine patterns and the final concentrations of protein in the tissues and were expressed in pg/mg.

Statistical Analyses

All statistical analyses were performed using the GraphPad Prism software (version 8.0.2). The data are presented as the mean ± standard error (SEM). Data normality was verified with the Kolmogorov-Smirnov test. Comparisons among all groups were made using two-way ANOVA. The Tukey's *post-hoc* analysis was used when the two-way ANOVA detected a statistical difference to assess multiple comparisons. The level of significance was $p < 0.05$.

RESULTS

Dietary Intervention, but Not Aerobic Exercise, Reduced Weight Gain in Obese Rats

To explore the role of aerobic exercise and dietary intervention in weight gain, Wistar rats were fed an HFD for 8 weeks and subject to aerobic exercise, diet switch, or both interventions

for an additional 8 weeks. The changes in body mass (g) are shown in **Table 1**. Regarding the baseline values (week 0) and after 8 weeks of obesity induction with HFD (week 8), the body mass did not differ between the groups ($p > 0.05$). After 8 weeks of dietary intervention and/or MICT (week 16), the body mass decreased in the CD-SED ($p = 0.002$) and CD-MICT groups ($p = 0.003$) compared to the HFD-SED group. The table also shows the variation in percentage (Δ%) between weeks 8 and 16. Similarly, the CD-SED and CD-MICT groups showed a significantly lower variation in percentage compared to the HFD-SED group ($p = 0.02$ and 0.002, respectively). These data suggest that the HFD was efficient in increasing the body mass of animals, and this process was reduced by dietary intervention independently and combined with MICT. Regarding food consumption, no differences were found in the daily consumption (g/day) between the groups ($p > 0.05$). Furthermore, the CD-SED group showed a lower caloric intake (kcal/day) compared to the HFD-SED group ($p = 0.009$) (**Table 1**).

Regarding the relative weights of depots (g/100 of body mass), in the mWAT depot, the CD-SED and CD-MICT groups showed lower values compared to the HFD-SED group ($p = 0.003$ and 0.009, respectively). For GAST, no statistically significant differences were found between the groups ($p > 0.05$). All the data are shown in **Table 2**.

Moderate-Intensity Continuous Training Associated With Dietary Intervention Promoted Adaptations in the Maximum Exercise Capacity Parameters in Greater Magnitudes

In the pre-intervention period, in the HFD-MICT group, the values of time ($p = 0.001$), distance ($p = 0.002$), and MEC ($p < 0.0001$) were higher compared to the CD-MICT group. For the variables time, distance, and MEC, both training groups showed an improvement in the parameters after 8 weeks of training ($p < 0.0001$). The delta percentage changes (Δ%) in MEC are also shown in the table. The CD-MICT group showed significantly greater improvements compared to the HFD-MICT group ($p = 0.001$). All the data are shown in **Table 3**.

Dietary Intervention, Independently and Combined With Moderate-Intensity Continuous Training, Reduced the Adipocyte Hypertrophy Caused by High-Fat Diet and Sedentarism

The values of the adipocyte diameter (μm^2) are shown in **Figure 1**. Similarly to body mass and the relative weight of mWAT depot, the CD-SED ($47.16 \pm 12.50\ \mu m^2$, $p = 0.01$) and CD-MICT ($37.59 \pm 8.19\ \mu m^2$, $p = 0.002$) groups showed a reduced adipocyte diameter compared to the HFD-SED group ($71.72 \pm 9.59\ \mu m^2$), with no difference in the group that received only MICT (HFD-MICT, $p = 0.58$). These data suggest that the dietary intervention, independently or combined with MICT, is effective in reducing the size of adipocytes caused by consumption of the HFD and by sedentary lifestyle.

Dietary Intervention, Independently and Associated With Moderate-Intensity Continuous Training, Was Efficient in Reducing the Concentration of Pro-inflammatory Markers in Mesenteric Adipose Tissue

The cytokine concentrations (pg/mg) in mWAT and GAST are shown in **Figure 2**. For mWAT, the CD-MICT group showed lower IL-6 values compared to the HFD-SED group ($p = 0.04$) (**Figure 2A**). For IL-10 in mWAT, the group that received the intervention with training independently (HFD-MICT) showed reduced values of this cytokine compared to the HFD-SED group ($p = 0.02$) (**Figure 2B**). In addition, the values of TNF-α were reduced in the group that performed the training independently (HFD-MICT, $p = 0.006$), dietary intervention (CD-SED, $p = 0.01$), and the combination of both interventions (CD-MICT, $p = 0.003$) compared to the HFD-SED group (**Figure 2C**). The results demonstrate that the reduction in pro-inflammatory cytokines (IL-6 and TNF-α) in mWAT may have occurred as a result of a reduction in body adiposity, as well as a reduction in the expansion of adipocytes caused by dietary intervention and a combination of both treatments. For GAST (**Figures 2D–F**), no statistically significant differences were found ($p > 0.05$).

Figure 3 shows the values of IL-10/TNF-α ratio. For mWAT (**Figure 3A**), the CD-MICT group showed higher values compared to the HFD-SED ($p = 0.01$), HFD-MICT ($p = 0.003$), and CD-SED groups ($p = 0.03$), demonstrating a change in the proportion of pro- vs. anti-inflammatory cytokine concentration in this tissue, caused by the combination of both interventions. In contrast, for GAST (**Figure 3B**), no statistical difference was found between the groups ($p > 0.05$).

DISCUSSION

This study reports the effects of MICT, dietary intervention (associated or not) on body mass, adipocyte expansion, and the modulation of inflammatory markers in diet-induced obese rats. Our results showed the dietary intervention, independently and combined with MICT, was able to reduce the body mass, adipocyte expansion, and relative weight of mWAT, and also generated positive adaptations in the concentrations of inflammatory markers in this tissue, whereas for GAST no changes were observed.

The effects of MICT and dietary intervention, associated or independently, on body mass and other obesity parameters have been extensively investigated. Regarding MICT, the results in the literature are still controversial; while some studies did not show positive changes on body mass with MICT (Martinez-Huenchullan et al., 2019; Khalafi et al., 2020), some of them have shown that this training model significantly reduced the body mass of obese animals (Wang et al., 2017). In the same

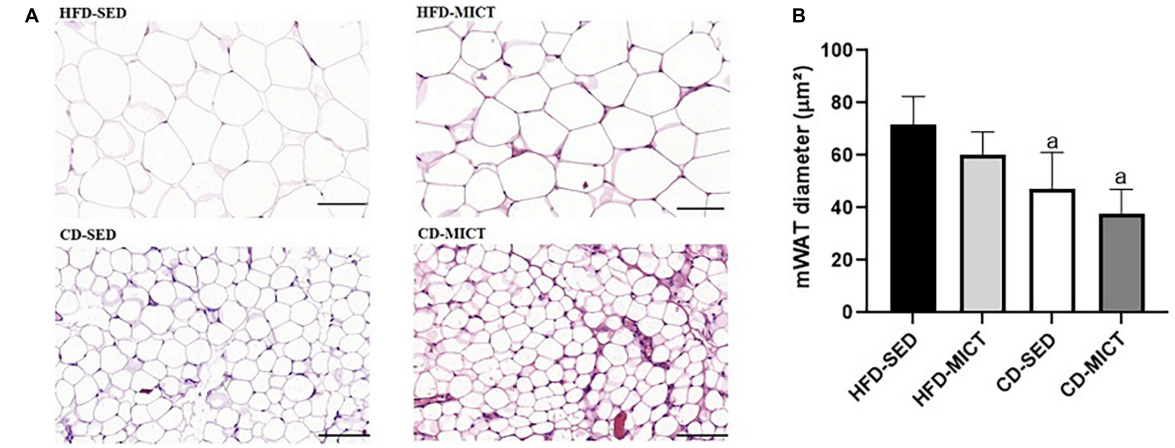

FIGURE 1 | Photomicrograph of mesenteric adipose tissue (mWAT) after 8 weeks of dietary intervention and/or MICT. **(A)** Representative images of mWAT tissue showing H&E staining using 20× (100 μm) objective. **(B)** Diameter of mWAT adipocytes. HFD-SED, high-fat diet sedentary (n = 5); HFD-MICT, high-fat diet plus MICT (n = 4); CD-SED, chow diet sedentary (n = 5); CD-MICT, chow diet plus MICT (n = 4). Data presented as mean ± SEM ($p < 0.05$). [a] vs. HFD-SED.

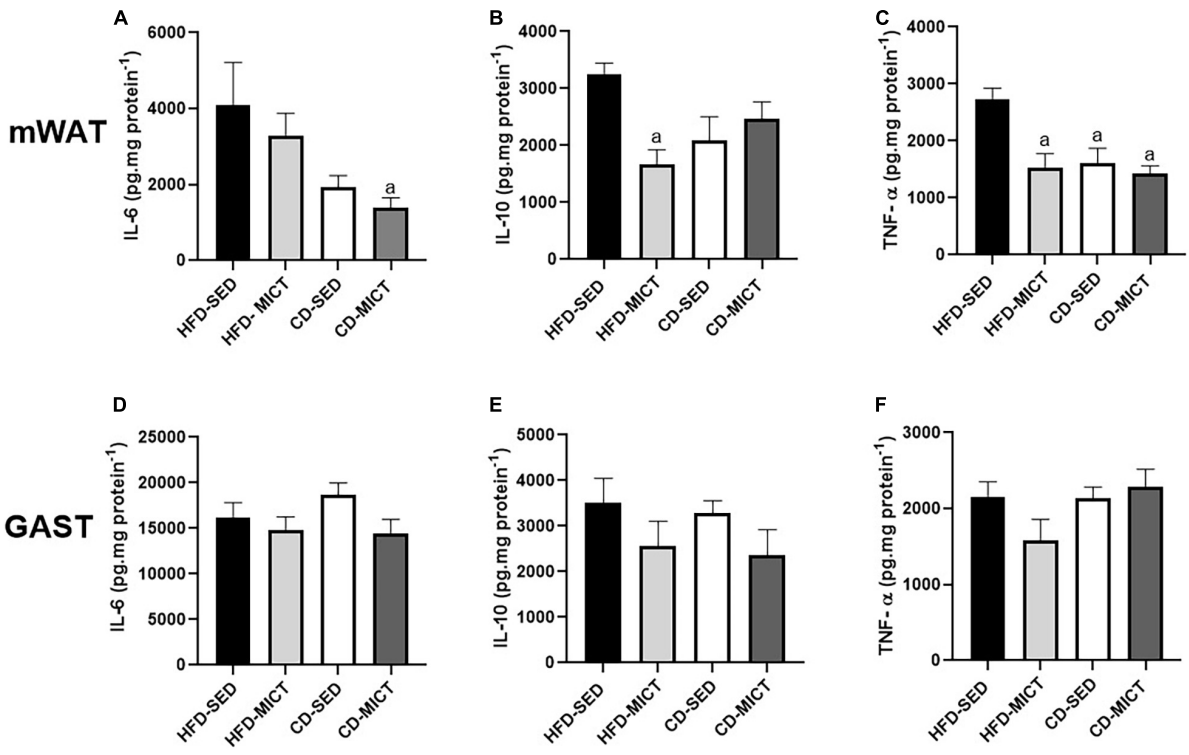

FIGURE 2 | Mesenteric adipose tissue (mWAT) and gastrocnemius muscle (GAST) cytokines concentration. **(A,D)** IL-6, interleukin-6; **(B,E)** IL-10, interleukin-10; **(C,F)** TNF-α, tumor necrosis factor alpha. HFD-SED, high-fat diet sedentary (n = 5); HFD-MICT, high-fat diet plus MICT (n = 5); CD-SED, chow diet sedentary (n = 5); CD-MICT, chow diet plus MICT (n = 5). Data presented as mean ± SEM ($p < 0.05$). [a] vs. HFD-SED.

way, MICT has independently been shown to be an important strategy to reduce adipocyte expansion (Lacerda et al., 2019; Khalafi et al., 2020).

In contrast to these previous studies, our findings indicated that MICT alone did not promote decreases in body mass, mWAT relative weight, and adipocyte hypertrophy of obese animals. Although a 30% reduction in the diameter of adipocytes of the HFD-MICT animals compared to the HFD-SED group was observed, this difference was not statistically significant (p = 0.44). These data can be associated with a reduced weekly training frequency in our protocol (three weekly sessions) compared to the protocols previously applied in the literature

(five weekly sessions, 65–70 of VO_{2max}) (Wang et al., 2017; Khalafi et al., 2020), suggesting that our protocol may not have increased in resting energy expenditure and resulted in a negative energy balance enough to generate such adaptations in adiposity (Lacerda et al., 2019; Khalafi et al., 2020). In addition, these results may be related to the fact that the mesenteric fat increases the lipogenesis *de novo* as a means to replace the lipids that are utilized during the exercise, hindering the loss of body mass and reduction in the area of adipocytes (Lehnig et al., 2019).

Furthermore, studies have shown that caloric restriction reduces body adiposity parameters due to the negative energy balance (Miller et al., 2017; Vangoitsenhoven et al., 2018). Moreover, the association between training and dietary interventions has a greater impact on body mass compared to the dietary intervention and exercise alone (Verheggen et al., 2016). Our results show that the dietary intervention used in this protocol (Δ14.2%) was effective in reducing the body mass, mWAT relative weight, and adipocyte size, with less marked changes in the feeding patterns. These results are reinforced by the literature, which showed that in diet-induced obese rats the consumption of 9% fat diet, or the return to a chow diet, was able to reduce the adipocyte size of obese rats (Vangoitsenhoven et al., 2018; Hansson et al., 2019).

Although no change in obesity parameters was observed in the HFD-MICT group, we also investigated other adaptations related to the treatments. In our study, both the MICT groups showed an increase in MEC compared to the pre-training results. However, the HFD-MICT group presented improvements in a smaller magnitude compared to the CD-MICT group. These results were previously attributed to the HFD effects on the metabolism of animals such as (a) a rapid accumulation of blood lactate due to a downregulation in lactate dehydrogenase isoform B (LDHB) and a decrease in monocarboxylate transporters 2 (MCT2) (Chen et al., 2017); (b) an alteration in glucose metabolism, which induces insulin resistance and glucose intolerance (Mardare et al., 2016); and (c) impairment of mitochondrial function in skeletal muscle (Takada et al., 2015). In our study, it is possible that these physiological aspects may have influenced the performance of the HFD-MICT group at the end of the experimental period, mainly on the deleterious effects of HFD on skeletal muscle (Ribeiro et al., 2019).

To consolidate the obese metabolic profile, we also evaluated the concentration of inflammatory cytokines. We observed that at 16 weeks of palatable HFD, increases in the concentration of inflammatory markers (IL-6, IL-10, and TNF-α) in the mWAT of the HFD-SED group were observed. These values were reduced after 8 weeks of dietary intervention (TNF-α), MICT (IL-10 and TNF-α), or both interventions (IL-6 and TNF-α). This behavior occurs, along with the reduction of body weight, adipocyte hypertrophy, and decrease in the relative weight of mWAT, and may be associated with an increase in lipolysis and a decrease in lipogenesis related to the irregular effect of these cytokines (e.g., IL-6 and TNF-α) during obesogenic processes (Jeong et al., 2015; Gonzalez-Gil and Elizondo-Montemayor, 2020).

Regarding pro-inflammatory cytokines, IL-10 presents an anti-inflammatory characteristic and plays an important role in the modulation of inflammatory processes during obesity,

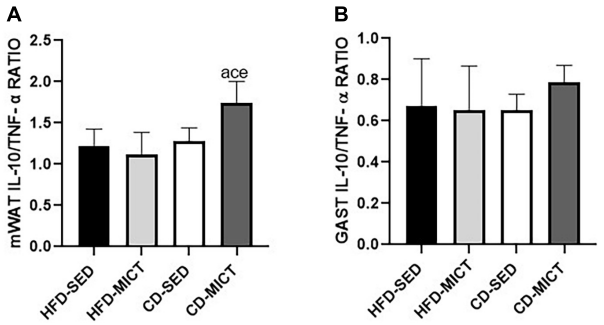

FIGURE 3 | IL-10/TNF-α ratio. **(A)** Mesenteric adipose tissue (mWAT). **(B)** Gastrocnemius muscle (GAST). HFD-SED, high-fat diet sedentary (n = 5); HFD-MICT, high-fat diet plus MICT (n = 5); CD-SED, chow diet sedentary (n = 5); CD-MICT, chow diet plus MICT (n = 5). Data presented as mean ± SEM ($p < 0.05$). [a] vs. HFD-SED; [c] vs. HFD- MICT; [e] vs. CD-SED.

mainly through the regulation and reduction of the inflammatory process caused by physical exercises (Lira et al., 2009a,b; Rocha-Rodrigues et al., 2017). Our findings showed that the concentration of IL-10 was reduced in the HFD-MICT group compared to the HFD-SED group. This higher concentration in the HFD-SED group may have been a consequence of the increase in the production of pro-inflammatory cytokines (e.g., TNF-α and IL-6) in order to minimize the inflammatory status (Esposito et al., 2003; Juge-Aubry et al., 2005; Yamashita et al., 2010). Furthermore, a decrease in the concentrations of this cytokine caused by exercises may have occurred for the same reason. These counterregulation effects occur because IL-10 can stimulate its own production, inhibiting the production of inflammatory cytokines (e.g., IL-1β, TNF-α, and IL-6) and also increasing the release of soluble TNF receptors, which antagonize the effects of TNF-α on adipose tissue (Yamashita et al., 2010).

Furthermore, the IL-10/TNF-α ratio was used in several studies as an indicator of the inflammatory status (Kaur et al., 2006; Leonidou et al., 2007; Jung et al., 2008; Lira et al., 2012). Previous studies have shown that aerobic training was able to promote increases in this ratio, both in serum (Chagas et al., 2017; Rocha-Rodrigues et al., 2017) and/or tissue levels (Lira et al., 2009a, 2012). On the other hand, our findings demonstrated that this effect resulted only due to the association of dietary intervention and MICT, suggesting that both interventions combined present an anti-inflammatory effect on mWAT. While in the GAST, since we did not see changes in cytokine concentrations, it was considered that the ratio would not change.

Furthermore, another mechanism that could explain the benefits of aerobic training in obese animals is the release of myokines. Some studies have reported the anti-inflammatory effects of physical exercise mediated by myokines, regardless of decreases in fat (Rosa-Neto et al., 2009; Balducci et al., 2012; Gopalan et al., 2021). In addition, exercising modalities such as running (Gopalan et al., 2021) or swimming (Shirvani et al., 2021) were shown to be important in reducing the inflammatory state of the muscle tissue due to the decrease in the deposition of ectopic fat in this compartment caused by training

or by the downregulation of the expression of TLR4/MyD88. The protocols and/or time of interventions in our study may have been insufficient to reduce fat deposition in this compartment because no differences were found in the relative muscle mass between the groups. However, due to the particularities of our protocols and the absence of a histological analysis to verify the deposition of fat in skeletal muscle, it is impossible to clearly affirm which mechanisms were involved in these processes.

This study has some limitations as follows: (1) the number of samples per group was limited to five animals. It is possible that a larger sample size would elicit stronger conclusions. (2) We did not perform histological analyses of GAST to check the deposition of ectopic fat in this tissue or serum concentrations of inflammatory or anti-inflammatory markers to add these parameters during our investigation. Hence, new perspectives for future studies with a larger number of animals per group, with other analysis and a longer time of intervention to investigate additional cytokines/myokines, are suggested. In conclusion, we found that the reduction in adiposity, the concentration of inflammatory markers in mWAT, and increases in MEC in greater magnitudes play a major role in the benefits induced by dietary intervention, combined or not with MICT, demonstrating that dietary intervention and the combination of both treatments are effective in reducing some of the deleterious effects caused by obesity.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Ethics Committee on the Use of Animals (CEUA) of Federal University of São Carlos (São Paulo, Brazil) approved all experimental procedures under protocol no. 7631210617.

AUTHOR CONTRIBUTIONS

JC, VF, and AD helped conceive the design, performed the analyses, analyzed the data, and wrote the first draft of the manuscript and helped conceive the design, helped with the data analyses, provided funding for the study, and helped draft the manuscript. JC, VF, and JA helped to conceive the design and supervised the experimental trials and training sessions. JC, VF, and CC helped with other data analyses and helped draft the manuscript. JC, VF, CC, JA, and AD interpreted the study results and edited the manuscript. All authors have read and approved the final version of the manuscript and agreed with the order of presentation of the authors.

FUNDING

This study was financed in part by the Coordenação de Aperfeiçoamento Pessoal de Nível Superior–Brazil (CAPES)–Finance Code 001, the National Council for Scientific and Technological Development CNPq (131213/2020-2), and grants from the São Paulo Research Foundation (FAPESP Grant #2017/09602-4). Funders played no role in the design, conduct, or reporting of this study.

REFERENCES

Aouichat, S., Chayah, M., Bouguerra-Aouichat, S., and Agil, A. (2020). Time-Restricted Feeding Improves Body Weight Gain, Lipid Profiles, and Atherogenic Indices in Cafeteria-Diet-Fed Rats: Role of Browning of Inguinal White Adipose Tissue. *Nutrients* 12:2185. doi: 10.3390/nu12082185

Balducci, S., Zanuso, S., Cardelli, P., Salvi, L., Mazzitelli, G., Bazuro, A., et al. (2012). Italian Diabetes Exercise Study (IDES) Investigators. Changes in physical fitness predict improvements in modifiable cardiovascular risk factors independently of body weight loss in subjects with type 2 diabetes participating in the Italian Diabetes and Exercise Study (IDES). *Diab. Care* 35, 1347–1354. doi: 10.2337/dc11-1859

Batatinha, H., Rosa Neto, J. C., and Krüger, K. (2019). Inflammatory features of obesity and smoke exposure and the immunologic effects of exercise. *Exerc. Immunol. Rev.* 25, 96–111.

Bay, M. L., and Pedersen, B. K. (2020). Muscle-Organ Crosstalk: Focus on Immunometabolism. *Front. Physiol.* 11:567881. doi: 10.3389/fphys.2020.567881

Bellicha, A., van Baak, M. A., Battista, F., Beaulieu, K., Blundell, J. E., Busetto, L., et al. (2021). Effect of exercise training on weight loss, body composition changes, and weight maintenance in adults with overweight or obesity: An overview of 12 systematic reviews and 149 studies. *Obes. Rev.* 22:e13256. doi: 10.1111/obr.13256

Bray, G. A., Frühbeck, G., Ryan, D. H., and Wilding, J. P. (2016). Management of obesity. *Lancet* 387, 1947–1956. doi: 10.1016/S0140-6736(16)00271-3

Cao, E., Watt, M. J., Nowell, C. J., Quach, T., Simpson, J. S., De Melo Ferreira, V., et al. (2021). Mesenteric lymphatic dysfunction promotes insulin resistance and represents a potential treatment target in obesity. *Nat. Metabol.* 3, 1175–1188. doi: 10.1038/s42255-021-00457-w

Chagas, E. F. B., Bonfim, M. R., Turi, B. C., Brondino, N. C. M., and Monteiro, H. L. (2017). Effect of Moderate-Intensity Exercise on Inflammatory Markers Among Postmenopausal Women. *J. Phys. Act. Health* 14, 479–485. doi: 10.1123/jpah.2016-0319

Chen, C. J., Liao, Y. H., Lin, S. Y., Yu, J. X., Li, Z. J., Lin, Y. C., et al. (2017). Diet-induced obesity accelerates blood lactate accumulation of rats in response to incremental exercise to maximum. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 313, R601–R607. doi: 10.1152/ajpregu.00337.201

Chouchani, E. T., and Kajimura, S. (2019). Metabolic adaptation and maladaptation in adipose tissue. *Nat. Metabol.* 1, 189–200. doi: 10.1038/s42255-018-0021-8

Costa, L. R., de Castro, C. A., Marine, D. A., Fabrizzi, F., Furino, V. O., Malavazi, I., et al. (2021). High-Intensity Interval Training Does Not Change Vaspin and Omentin and Does Not Reduce Visceral Adipose Tissue in Obese Rats. *Front. Physiol.* 12:564862. doi: 10.3389/fphys.2021.564862

Esposito, K., Pontillo, A., Giugliano, F., Giugliano, G., Marfella, R., Nicoletti, G., et al. (2003). Association of low interleukin-10 levels with the metabolic syndrome in obese women. *J. Clin. Endocrinol. Metab.* 88, 1055–1058. doi: 10.1210/jc.2002-021437

Estadella, D., Oyama, L. M., Dâmaso, A. R., Ribeiro, E. B., Oller, and Do Nascimento, C. M. (2004). Effect of palatable hyperlipidic diet on lipid metabolism of sedentary and exercised rats. *Nutrition* 20, 218–224. doi: 10.1016/j.nut.2003.10.008

Furino, V. O., Alves, J. M., Marine, D. A., Sene-Fiorese, M., Rodrigues, C., Arrais-Lima, C., et al. (2021). Dietary Intervention, When Not Associated

with Exercise, Upregulates Irisin/FNDC5 While Reducing Visceral Adiposity Markers in Obese Rats. *Front. Physiol.* 12:564963. doi: 10.3389/fphys.2021.564963

Galarraga, M., Campión, J., Muñoz-Barrutia, A., Boqué, N., Moreno, H., Martínez, J. A., et al. (2012). Adiposoft: automated software for the analysis of white adipose tissue cellularity in histological sections. *J. Lipid. Res.* 53, 2791–2796. doi: 10.1194/jlr.D023788

Gonzalez-Gil, A. M., and Elizondo-Montemayor, L. (2020). The Role of Exercise in the Interplay between Myokines, Hepatokines, Osteokines, Adipokines, and Modulation of Inflammation for Energy Substrate Redistribution and Fat Mass Loss: A Review. *Nutrients* 12:1899. doi: 10.3390/nu12061899

Gopalan, V., Yaligar, J., Michael, N., Kaur, K., Anantharaj, R., Verma, S. K., et al. (2021). A 12-week aerobic exercise intervention results in improved metabolic function and lower adipose tissue and ectopic fat in high-fat diet fed rats. *Biosci. Rep.* 41:BSR20201707. doi: 10.1042/BSR20201707

Han, M. S., White, A., Perry, R. J., Camporez, J. P., Hidalgo, J., Shulman, G. I., et al. (2020). Regulation of adipose tissue inflammation by interleukin 6. *Proc. Natl. Acad. Sci. U.S.A.* 117, 2751–2760. doi: 10.1073/pnas.1920004117

Hansson, B., Morén, B., Fryklund, C., et al. (2019). Adipose cell size changes are associated with a drastic actin remodeling. *Sci. Rep.* 9:12941. doi: 10.1038/s41598-019-49418-0

Jais, A., and Brüning, J. C. (2017). Hypothalamic inflammation in obesity and metabolic disease. *J. Clin. Invest.* 127, 24–32. doi: 10.1172/JCI88878

Jeong, J. H., Park, H. G., Lee, Y. R., and Lee, W. L. (2015). Moderate exercise training is more effective than resveratrol supplementation for ameliorating lipid metabolic complication in skeletal muscle of high fat diet-induced obese mice. *J. Exerc. Nutr. Biochem.* 19, 131–137. doi: 10.5717/jenb.2015.15062211

Juge-Aubry, C. E., Somm, E., Pernin, A., Alizadeh, N., Giusti, V., Dayer, J. M., et al. (2005). Adipose tissue is a regulated source of interleukin-10. *Cytokine* 29, 270–274. doi: 10.1016/j.cyto.2004.10.017

Jung, S. H., Park, H. S., Kim, K. S., Choi, W. H., Ahn, C. W., Kim, B., et al. (2008). Effect of weight loss on some serum cytokines in human obesity: increase in IL-10 after weight loss. *J. Nutr. Biochem.* 19, 371–375. doi: 10.1016/j.jnutbio.2007.05.007

Kaur, K., Sharma, A. K., Dhingra, S., and Singal, P. K. (2006). Interplay of TNF-alpha and IL-10 in regulating oxidative stress in isolated adult cardiac myocytes. *J. Mol. Cell Cardiol.* 41, 1023–1030. doi: 10.1016/j.yjmcc.2006.08.005

Kawai, T., Autieri, M. V., and Scalia, R. (2021). Adipose tissue inflammation and metabolic dysfunction in obesity. *Am. J. Physiol. Cell Physiol.* 320, C375–C391. doi: 10.1152/ajpcell.00379.2020

Khalafi, M., Mohebbi, H., Symonds, M. E., Karimi, P., Akbari, A., Tabari, E., et al. (2020). The Impact of Moderate-Intensity Continuous or High-Intensity Interval Training on Adipogenesis and Browning of Subcutaneous Adipose Tissue in Obese Male Rats. *Nutrients* 12:925. doi: 10.3390/nu12040925

Kunstetter, A. C., Barbosa, N., Moraes, M. M., Pinto, V. A., Soares, D. D., Pires, et al. (2018). Pre-exercise exposure to the treadmill setup changes the cardiovascular and thermoregulatory responses induced by subsequent treadmill running in rats. *Temperature* 5, 109–122. doi: 10.1080/23328940.2017.1388343

Lacerda, D. R., Moraes, M. M., Nunes-Silva, A., Costa, K. A., Rodrigues, D. F., Sabino, J. L., et al. (2019). Aerobic training reduces immune cell recruitment and cytokine levels in adipose tissue in obese mice. *Appl. Physiol. Nutr. Metab.* 5, 512–520. doi: 10.1139/apnm-2018-0523

Lancaster, G. I., and Febbraio, M. A. (2014). The immunomodulating role of exercise in metabolic disease. *Trends Immunol.* 35, 262–269. doi: 10.1016/j.it.2014.02.008

Leal, L. G., Lopes, M. A., and Batista, M. L. Jr. (2018). Physical Exercise-Induced Myokines and Muscle-Adipose Tissue Crosstalk: A Review of Current Knowledge and the Implications for Health and Metabolic Diseases. *Front. Physiol.* 9:1307. doi: 10.3389/fphys.2018.01307

Lehnig, A. C., Dewal, R. S., Baer, L. A., Kitching, K. M., Munoz, V. R., Arts, P. J., et al. (2019). Exercise Training Induces Depot-Specific Adaptations to White and Brown Adipose Tissue. *iScience* 11, 425–439. doi: 10.1016/j.isci.2018.12.033

Leonidou, L., Mouzaki, A., Michalaki, M., DeLastic, A. L., Kyriazopoulou, V., Bassaris, H. P., et al. (2007). Cytokine production and hospital mortality in patients with sepsis-induced stress hyperglycemia. *J. Infect.* 55, 340–346. doi: 10.1016/j.jinf.2007.05.177

Lira, F. S., Rosa, J. C., Yamashita, A. S., Koyama, C. H., Batista, M. L. Jr., and Seelaender, M. (2009a). Endurance training induces depot-specific changes in IL-10/TNF-alpha ratio in rat adipose tissue. *Cytokine* 45, 80–85. doi: 10.1016/j.cyto.2008.10.018

Lira, F. S., Rosa, J. C., Zanchi, N. E., Yamashita, A. S., Lopes, R. D., Lopes, A. C., et al. (2009b). Regulation of inflammation in the adipose tissue in cancer cachexia: effect of exercise. *Cell Biochem. Funct.* 27, 71–75. doi: 10.1002/cbf.1540

Lira, F. S., Yamashita, A. S., Rosa, J. C., Koyama, C. H., Caperuto, E. C., Batista, M. L. Jr., et al. (2012). Exercise training decreases adipose tissue inflammation in cachectic rats. *Horm. Metab. Res.* 44, 91–98. doi: 10.1055/s-0031-1299694

Longo, V. D., and Panda, S. (2016). Fasting. Circadian Rhythms, and Time-Restricted Feeding in Healthy Lifespan. *Cell Metab.* 23, 1048–1059. doi: 10.1016/j.cmet.2016.06.001

Lowe, D. A., Wu, N., Rohdin-Bibby, L., Moore, A. H., Kelly, N., Liu, Y. E., et al. (2020). Effects of Time-Restricted Eating on Weight Loss and Other Metabolic Parameters in Women and Men with Overweight and Obesity: The TREAT Randomized Clinical Trial. *JAMA Internal Med.* 180, 1491–1499. doi: 10.1001/jamainternmed.2020.4153

Mardare, C., Krüger, K., Liebisch, G., Seimetz, M., Couturier, A., Ringseis, R., et al. (2016). Endurance and Resistance Training Affect High Fat Diet-Induced Increase of Ceramides, Inflammasome Expression, and Systemic Inflammation in Mice. *J. Diab. Res.* 2016:4536470. doi: 10.1155/2016/4536470

Martinez-Huenchullan, S. F., Ban, L. A., Olaya-Agudo, L. F., et al. (2019). Constant-Moderate and High-Intensity Interval Training Have Differential Benefits on Insulin Sensitive Tissues in High-Fat Fed Mice. *Front. Physiol.* 10:459. doi: 10.3389/fphys.2019.00459

Miller, K. N., Burhans, M. S., Clark, J. P., Howell, P. R., Polewski, M. A., DeMuth, T. M., et al. (2017). Aging and caloric restriction impact adipose tissue, adiponectin, and circulating lipids. *Aging Cell* 16, 497–507. doi: 10.1111/acel.12575

Parlee, S. D., Lentz, S. I., Mori, H., and MacDougald, O. A. (2014). Quantifying size and number of adipocytes in adipose tissue. *Methods Enzymol.* 014, 93–122. doi: 10.1016/B978-0-12-411619-1.00006-9

Pedersen, B. K., Akerström, T. C., Nielsen, A. R., and Fischer, C. P. (2007). Role of myokines in exercise and metabolism. *J. Appl. Physiol.* 103, 1093–1098. doi: 10.1152/japplphysiol.00080.2007

Rachetti, A. L., Arida, R. M., Patti, C. L., Zanin, K. A., Fernades-Santos, L., Frussa-Filho, et al. (2013). Fish oil supplementation and physical exercise program: distinct effects on different memory tasks. *Behav. Brain Res.* 237, 283–289. doi: 10.1016/j.bbr.2012.09.048

Ribeiro, F. M., Ribeiro, C. F. A., Acm, G., Castro, A. P., Almeida, J. A., Franco, O. L., et al. (2019). Limited Effects of Low-to-Moderate Aerobic Exercise on the Gut Microbiota of Mice Subjected to a High-Fat Diet. *Nutrients* 11:149. doi: 10.3390/nu11010149

Rocha-Rodrigues, S., Rodríguez, A., Gonçalves, I. O., Moreira, A., Maciel, E., Santos, S., et al. (2017). Impact of physical exercise on visceral adipose tissue fatty acid profile and inflammation in response to a high-fat diet regimen. *Int. J. Biochem. Cell Biol.* 87, 114–124. doi: 10.1016/j.biocel.2017.04.008

Rosa-Neto, J. C., Lira, F. S., Oyama, L. M., Zanchi, N. E., Yamashita, A. S., Batista, M. L. Jr., et al. (2009). Exhaustive exercise causes an anti-inflammatory effect in skeletal muscle and a pro-inflammatory effect in adipose tissue in rats. *Eur. J. Appl. Physiol.* 106, 697–704. doi: 10.1007/s00421-009-1070-1

Shirvani, H., Mirnejad, R., Soleimani, M., and Arabzadeh, E. (2021). Swimming exercise improves gene expression of PPAR-γ and downregulates the overexpression of TLR4, MyD88, IL-6, and TNF-α after high-fat diet in rat skeletal muscle cells. *Gene* 775:145441. doi: 10.1016/j.gene.2021.145441

Souza, R., Alves, C., Medeiros, A., Rolim, N., Silva, G., Moreira, J., et al. (2018). Differential regulation of cysteine oxidative post-translational modifications in high and low aerobic capacity. *Scient. Rep.* 8:17772. doi: 10.1038/s41598-018-35728-2

Steensberg, A., Fischer, C. P., Keller, C., Møller, K., and Pedersen, B. K. (2003). IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. American journal of physiology. *Endocrinol. Metab.* 285, E433–E437. doi: 10.1152/ajpendo.00074.2003

Steensberg, A., van Hall, G., Osada, T., Sacchetti, M., Saltin, B., and Pedersen, B. (2000). Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. *J. Physiol.* 2529, 237–242. doi: 10.1111/j.1469-7793.2000.00237.x

Takada, S., Kinugawa, S., Matsushima, S., Takemoto, D., Furihata, T., Mizushima, W., et al. (2015). Sesamin prevents decline in exercise capacity and impairment

of skeletal muscle mitochondrial function in mice with high-fat diet-induced diabetes. *Exp. Physiol.* 100, 1319–1330. doi: 10.1113/EP085251

Vangoitsenhoven, R., van der Ende, M., Corbeels, K., Monteiro Carvalho, J. P., Lannoo, M., et al. (2018). At similar weight loss, dietary composition determines the degree of glycemic improvement in diet-induced obese C57BL/6 mice. *PLoS One.* 13:e0200779. doi: 10.1371/journal.pone.0200779

Verheggen, R. J., Maessen, M. F., Green, D. J., Hermus, A. R., Hopman, M. T., and Thijssen, D. H. (2016). A systematic review and meta-analysis on the effects of exercise training versus hypocaloric diet: distinct effects on body weight and visceral adipose tissue. *Obes. Rev.* 17, 664–690. doi: 10.1111/obr.12406

Wang, N., Liu, Y., Ma, Y., and Wen, D. (2017). High-intensity interval versus moderate-intensity continuous training: Superior metabolic benefits in diet-induced obesity mice. *Life Sci.* 191, 122–131. doi: 10.1016/j.lfs.2017.08.023

Wewege, M., van den Berg, R., Ward, R. E., and Keech, A. (2017). The effects of high-intensity interval training vs. moderate-intensity continuous training on body composition in overweight and obese adults: a systematic review and meta-analysis. *Obes. Rev.* 18, 635–646. doi: 10.1111/obr.12532

Yamashita, A. S., Lira, F. S., Rosa, J. C., Paulino, E. C., Brum, P. C., Negrão, C. E., et al. (2010). Depot-specific modulation of adipokine levels in rat adipose tissue by diet-induced obesity: the effect of aerobic training and energy restriction. *Cytokine* 52, 168–174. doi: 10.1016/j.cyto.2010.07.006

Zatterale, F., Longo, M., Naderi, J., Raciti, G. A., Desiderio, A., Miele, C., et al. (2020). Chronic Adipose Tissue Inflammation Linking Obesity to Insulin Resistance and Type 2 Diabetes. *Front. Physiol.* 10:1607. doi: 10.3389/fphys.2019.01607

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frontiers | Frontiers in Physiology

ORIGINAL RESEARCH
published: 26 April 2022
doi: 10.3389/fphys.2022.803126

Melatonin Potentiates Exercise-Induced Increases in Skeletal Muscle PGC-1α and Optimizes Glycogen Replenishment

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Compelling evidence has demonstrated the effect of melatonin on exhaustive exercise tolerance and its modulatory role in muscle energy substrates at the end of exercise. In line with this, PGC-1α and NRF-1 also seem to act on physical exercise tolerance and metabolic recovery after exercise. However, the literature still lacks reports on these proteins after exercise until exhaustion for animals treated with melatonin. Thus, the aim of the current study was to determine the effects of acute melatonin administration on muscle PGC-1α and NRF-1, and its modulatory role in glycogen and triglyceride contents in rats subjected to exhaustive swimming exercise at an intensity corresponding to the anaerobic lactacidemic threshold (iLAn). In a randomized controlled trial design, thirty-nine Wistar rats were allocated into four groups: control (CG = 10), rats treated with melatonin (MG = 9), rats submitted to exercise (EXG = 10), and rats treated with melatonin and submitted to exercise (MEXG = 10). Forty-eight hours after the graded exercise test, the animals received melatonin (10 mg/kg) or vehicles 30 min prior to time to exhaustion test in the iLAn (*t*lim). Three hours after *t*lim the animals were euthanized, followed by muscle collection for specific analyses: soleus muscles for immunofluorescence, gluteus maximus, red and white gastrocnemius for the assessment of glycogen and triglyceride contents, and liver for the measurement of glycogen content. Student t-test for independent samples, two-way ANOVA, and Newman keuls post hoc test were used. MEXG swam 120.3% more than animals treated with vehicle (EXG; $p < 0.01$). PGC-1α and NRF-1 were higher in MEXG with respect to the CG ($p < 0.05$); however, only PGC-1α was higher for MEXG when compared to EXG. Melatonin reduced the triglyceride content in gluteus maximus, red and white gastrocnemius ($F = 6.66$, $F = 4.51$, and $F = 6.02$, $p < 0.05$). The glycogen content in red gastrocnemius was higher in MEXG than in CG ($p = 0.01$), but not in EXG ($p > 0.05$). In conclusion, melatonin was found to enhance exercise tolerance, potentiate exercise-mediated increases in PGC-1α, decrease muscle triglyceride content and increase muscle

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Edited by:
Ana Maria Teixeira, University of Coimbra, Portugal

Reviewed by:
Guilherme Eustaquio Furtado, Instituto Politécnico da Guarda, Portugal
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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: *27 October 2021*
Accepted: *03 March 2022*
Published: *26 April 2022*

Citation:
Faria VS, Manchado-Gobatto FB, Scariot PPM, Zagatto AM and Beck WR (2022) Melatonin Potentiates Exercise-Induced Increases in Skeletal Muscle PGC-1α and Optimizes Glycogen Replenishment. Front. Physiol. 13:803126. doi: 10.3389/fphys.2022.803126

Abbreviations: ES, Effect size; GXT, graded exercise test; iLAn, intensity of anaerobic lactacidemic threshold; PGC-1α KO, whole-body PGC-1α knockout animals; PGC-1α MKO, skeletal muscle-specific PGC-1α knockout.

glycogen 3 h after exhaustive exercise, rapidly providing a better cellular metabolic environment for future efforts.

Keywords: peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), nuclear respiratory factor 1 (NRF-1), energy metabolism (MeSH ID: D004734), aerobic exercise, N-acetyl-5-methoxytryptamine, recovery, ergogenic aid, skeletal muscle tissue

1 INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is considered an indoleamine with amphiphilic characteristics (Amaral et al., 2019). Although it is mainly produced by the pineal gland and directly released into the blood or cerebrospinal fluid, melatonin is also found in several extra pineal tissues, including the brain, retina, liver, skeletal muscle, and so on (Acuña-Castroviejo et al., 2014). Substantial evidence has confirmed the regulatory role of melatonin in circadian and seasonal rhythms (Reiter et al., 2010; Brzezinski et al., 2021; Pevet et al., 2021; Hardeland, 2022), antioxidant (Rodriguez et al. al, 2004; Manchester et al., 2015; Reiter et al., 2016; Ortiz-Franco et al., 2017; Galano et al., 2018; Chitimus et al., 2020; Kruk et al., 2021) and anti-inflammatory effects (Mauriz et al., 2013) among others. In addition to these benefits, our research group has demonstrated the ergogenic effect of melatonin in the increasing exhaustive aerobic exercise tolerance of nocturnal animals (Beck et al., 2015A; Beck et al., 2016; Faria et al., 2021), whereas the effect of melatonin on metabolic recovery after physical exercise is less reported. Specifically, the role of melatonin in muscle glycogen and triglyceride contents few hours after exercise is not fully understood. Additionally, little attention has been given to the role of melatonin administration on PGC-1α and NRF-1, which are considered representatives of the aerobic energy metabolism.

In this scenario, we sought to study proteins related to aerobic adaptations that could improve the mitochondrial capacity and possibly modulate the content of energy substrates in the skeletal muscle tissue after an exercise session. Regarding the proteins PGC-1α and NRF-1, the peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) is a transcriptional coactivator that interacts with nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) (Lira et al., 2010; Islam et al., 2018; Islam et al., 2020) to stimulate the mitochondrial biogenesis and function (Bonen, 2009; Seebacher and Glanville, 2010; Islam et al., 2018; Islam et al., 2020). In addition, PGC-1α seems to act on the energy metabolism through glycogen content increase (Wende et al., 2007; Wong et al., 2015) and fatty acid oxidation (Wong et al., 2015), with a concomitant enhancement in exhaustive exercise tolerance (Tadaishi et al., 2011; Wong et al., 2015). However, in an opposite scenario of high PGC-1α, there are evidences showing a lower endurance performance in skeletal muscle-specific PGC-1α knockout (PGC-1α MKO) (Handschin et al., 2007) and whole-body PGC-1α knockout animals (PGC-1α KO) (Leone et al., 2005). Although it is well established that a single bout of exercise is able to increase content and/or expression of PGC-1α (Wright et al., 2007; Ikeda et al., 2008; Seebacher and Glanville, 2010; Fujimoto et al., 2011; Shute et al., 2018) and NRF-1 (Murakami et al., 1998; Seebacher and Glanville, 2010; Daussin et al., 2012), reports on these proteins after exercise until exhaustion are lacking for animals treated with melatonin.

Based on the aforementioned assumptions, the literature demonstrates a carbohydrate dependence during prolonged exercise (Leckey et al., 2016; Burke and Hawley, 2018; Hargreaves and Spriet, 2020), establishing that its reduction is a limiting factor for performance (Krssak et al., 2000). Hence, a rapid glycogen repletion following a bout of exhaustive exercise is an important adaptative response to prepare the muscle for subsequent efforts (Wende et al., 2007), at least from a bioenergetic point of view. Moreover, to produce a better exercise training strategy it is essential to understand the beneficial effects of a single bout of exercise since training adaptations reflect the accumulation of beneficial physiological functions produced from acute exercise (Park et al., 2021). Even though some studies suggest that melatonin exerts a modulatory role in muscle energy substrates immediately at the end of exercise (Mazepa et al., 2000; Sanchez-Campos et al., 2001), decreasing carbohydrate utilization and increasing lipid utilization (Mazepa et al., 2000), no study has investigated the effect of melatonin on the recovery of muscle glycogen and triglyceride contents at later times after an exhaustive bout of exercise at an individual and objective intensity. Therefore, the current study aims to determine the effects of acute administration of melatonin on exercise tolerance, glycogen and/or triglyceride contents in the skeletal muscle and liver, as well as PGC-1α and NRF-1 expressions in the skeletal muscle of control rats (non-exercised) and rats subjected to exhaustive swimming exercise at an intensity corresponding to the anaerobic lactacidemic threshold (iLAn). We hypothesize that acute melatonin administration increases PGC-1α, NRF-1, and muscle glycogen content, in addition to reducing muscle triglyceride content in exercised skeletal muscle, consequently providing a better cellular environment for future efforts.

2 MATERIALS AND METHODS

2.1 Animals and Environmental Conditions

Forty young male Wistar rats (45 days old at arrival; weighing between 120 and 150 g) were provided by the Central Animal Facility of the Federal University of São Carlos—UFSCar (Brazil). The animals were housed in controlled environmental conditions: temperature (22 ± 2°C), relative humidity (between 45 and 55%), noise (<85 dB), and photoperiod (10:14 h light/dark cycle), as suggest by the guidelines for the housing of rats in scientific institutions (ARRP Guideline 20). Animals (4–5 per cage) received commercial chow and filtered water *ad libitum*. As

albino animals are easily affected by phototoxic retinopathy (ARRP, 2007; Castelhano-Carlos and Baumans, 2009), a stressful condition capable of generating undesirable interference in experiments, especially those involved with melatonin and circadian rhythm, incandescent lamps (Philips, model Soft, 100 W, 2700 K; 565–590 nm; 60 lux, measured by a lux meter) were used during the 10-hour light cycle. To carry out experimental interventions with the rats during the dark cycle (nighttime: 4:00 p.m. to 6:00 a.m.), reflectors were installed around a red filter (ROSCO, model # fire19; > 600 nm; < 15 lux) (Beck and Gobatto, 2013; Beck et al., 2015B; Beck et al., 2016; Faria et al., 2021). Such a luminous scenario makes it possible to prevent the relevant influence of light on the activity of N-acetyltransferase in the pineal gland (Sun et al., 1993), an extremely important enzyme for melatonin biosynthesis. All experimental procedures were conducted in accordance with the Ethical Principles in Animal Research (ARRIVE guidelines 2.0), adopted by the Brazilian College of Animal Experimentation (COBEA, Brazil), and were approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of São Carlos—UFSCar (São Paulo, Brazil) under protocol no. 9144181218.

2.2 Experimental Design

In a randomized controlled trial design, the rats ($n = 39$) were divided into four groups: control (CG: $n = 10$), treated with melatonin (MG: $n = 9$), submitted to exercise (EXG: $n = 10$), and treated with melatonin and submitted to exercise (MEXG: $n = 10$). These groups originally numbered 10 animals each, however, throughout the experiment we lost one of them with no defined cause. The animals in the CG and EXG received vehicle solution (ethanol and NaCl, 0.9%), while those belonging to the MG and MEXG received melatonin (10 mg/kg) 30 min before the time to exhaustion test, being euthanized 3 h after the end of the exercise session.

2.3 Adaptation to Aquatic Environment and Swimming

After environmental adaptation (from 76 to 89 days old) all rats were adapted to aquatic environment and swimming exercise, considering a protocol adapted from Lima et al. (2017). Initially, the animals were submitted to the aquatic environment in shallow water (10 cm) for 5 min, with increments of 5 min per day for 3 days. Then, the rats were exposed to the swimming exercise protocol in deep water (80 cm) for 2 min in 2-minute increments per day for 7 days. Subsequently, the animals were submitted to swimming exercise in deep water (80 cm) with a load weight of 3% of body mass (attached to the animal's back) for 5 min, with increments of 5 min per day for 4 days. The animals were introduced to individual swimming protocols in cylindrical and opaque tanks—height: 100 cm (water depth: 80 cm), diameter: 30 cm, and water temperature: 31 ± 1°C, following the guidelines of the American Physiological Society (APS, 2006).

2.4 Graded Exercise Test

At 90 days old, all animals were subjected to the graded exercise test (GXT) to determine the exercise intensity corresponding to the individual anaerobic lactacidemic threshold (iLAn). According Beck et al. (2015B), iLAn is found when a disproportionate increase in the concentration of blood lactate is observed with respect to proportional increases in the intensity (imposed through loads corresponding to % of the body mass of each animal), here denominated as the anaerobic lactacidemic threshold. Thus, the animals were subjected to 5-min stages with overloads corresponding to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, and 7.0% of the body mass (% BM) attached to the animal's back through elastic strap. Each stage was separated by 30-second intervals in which blood samples (25 μL) were collected from the tip of the animals' tail and then stored (4°C) to determine lactatemia. The blood lactate concentration was plotted against exercise intensity on a scatter plot, and any change in the blood lactate concentration was identified by visual inspection, as previously described by Matsumoto et al. (1999). Then, two linear regressions were constructed following the break point, and the intersection of these linear regressions interpolated to the *X* axis was used to define the intensity corresponding to the anaerobic lactacidemic threshold (Beck et al., 2015B). The interpolation to *Y* axis corresponded to the blood lactate concentration in the iLAn.

2.5 Melatonin Administration

Melatonin (Sigma Aldrich Chemical Corporation; St Louis, MO, United States; M-5250, > 98%) was dissolved in ethanol (< 0.1%) and diluted in saline (0.9% NaCl) for administration at 10 mg/kg (Beck et al., 2015A; Beck et al., 2016; Faria et al., 2021). The preparation was performed prior to its use and stored in an amber bottle wrapped in aluminum foil. Its administration was made intraperitoneally 30 min prior to the time to exhaustion test.

2.6 Time to Exhaustion Test (*t*lim)

At 92 days old, 30 min after receiving melatonin (MG and MEXG) or vehicle (CG and EXG) the animals from EXG and MEXG were submitted to swimming exercise until exhaustion in the iLAn, the so-called time to exhaustion test (*t*lim). Then, the animals were introduced to the individual swimming protocol, and the time to exhaustion was recorded. The criterion for identifying the animal's exhaustion was standardized according to Beck and Gobatto (2013). To this end, the swimming behavior was analyzed in order to observe the execution of vigorous efforts without success in returning to the surface for a period of 15 s. The exhaustion was established by consensus of two experienced observers considering the above criteria. The timeline of events of animals aged from 90 to 92 days old is detailed in **Figure 1**.

2.7 Euthanasia, Obtention and Processing of Biological Materials

All animals were euthanized 3 h after the end of the experimental procedures by decapitation in agreement with the guidelines of the American Veterinary Medical Association (2013). Then, the skeletal muscle tissue (soleus, gluteus maximus, red and white portion of gastrocnemius), and the liver were collected, immediately frozen in liquid nitrogen and stored at −80°C for further analyses.

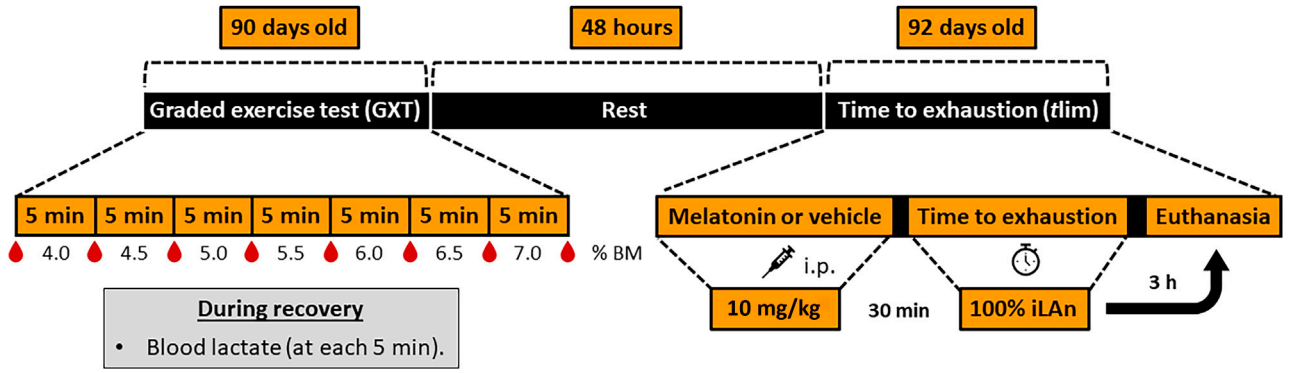

FIGURE 1 | Timeline of events of animals aged from 90 to 92 days old. After adaptation to aquatic environment procedures, at 90 days old all animals were subjected to graded exercise test (GXT) to determine the intensity of exercise corresponding to the individual anaerobic lactacidemic threshold (iLAn). During the GXT, the stages were separated by 30-second intervals in which blood samples (25 μL) were collected. Afterwards, the animals were submitted to the swimming exercise with increments of 0.5% of body mass (% BM) until exhaustion. Forty-eight hours later (rest period), the animals received melatonin (MG and MEXG: 10 mg/kg) or vehicle (CG and EXG: NaCl 0.09%), and after 30 min the rats from EXG and MEXG were submitted to the time to exhaustion test (*t*lim) at 100% of iLAn. After 3 h the animals were euthanized. h, hours; min, minutes; i.p., intraperitoneal.

2.7.1 Determination of Blood Lactate Concentration by an Enzymatic Assay

During the GXT, blood samples (25 μL) were collected from the animals' tails in heparinized and calibrated glass capillaries. Samples were placed inside plastic tubes (1.5 ml) containing 400 μL of trichloroacetic acid (4%), mixed and stored at 4°C. After stirring and centrifugation (3,000 rpm for 3 min), 50 μL of supernatant was extracted and transferred to a 96-well microplate, where 250 μL of reactive solution prepared for immediate use were added. This reactive solution was comprised of glycine, EDTA, and hydrazine 25%, and after pH adjustment to 9.45, β- nicotinamide adenine dinucleotide (NAD), and L-lactic dehydrogenase bovine heart (LDH) were incorporated to the resulting solution. After an incubation period of 20 min at 37°C, the samples were subjected to spectrophotometric measurements (Spectramax i3, Molecular Devices; San José, CA, United States) at 340 nm to compare the sample values to a standard curve constructed from a serial dilution of 1–15 mmol/L of L-Lactate.

2.7.2 Determination of Glycogen Content

The glycogen content within the skeletal muscle (gluteus maximus, red and white portion of gastrocnemius) and the liver was determined as described in Dubois et al. (1956). Both skeletal muscle (250 mg) and liver (500 mg) were firstly immersed in potassium hydroxide (30%; Êxodo Científica; Sumaré, SP, Brazil), and then mixed with saturated sodium sulfate solution (20 μL; Dinâmica Química Contemporânea Ltda; Indaiatuba, SP, Brazil) and ethanol [70%] for glycogen precipitation. The samples were homogenized with phenol (10 μL; Êxodo Científica; Sumaré, SP, Brazil) and sulfuric acid (2 ml; Dinâmica Química Contemporânea Ltda; Indaiatuba, SP, Brazil), and heated in water bath for 5 min (85°C). Finally, the absorbance was measured on a spectrophotometer (Hach Company, Loveland, Colo, United States; 490 nm), and the glycogen content was calculated using a calibration glucose curve.

2.7.3 Determination of Triglyceride Content

To determine the triglyceride content, the skeletal muscle (100–200 mg; gluteus maximus, red and white portion of gastrocnemius) was placed inside plastic tubes (1.5 ml) containing Triton X-100 [1%] at the same proportions (200 mg of tissue to 1 ml of Triton). Next, the samples were homogenized using magnetic bars (5 × 3 mm) overnight (4°C). After this period, the samples were centrifuged (4,000 rpm for 10 min), and 10 μL of the supernatant was extracted, pipetted onto a 96-well microplate in a mixture with the kit reagent (200 μL; LaborLab; Guarulhos, SP, Brazil) and incubated for 20 min (25°C). The triglyceride absorbance was performed on a spectrophotometer (SpectraMax i3, Molecular Devices; San José, CA, United States) at 505 nm, according to the kit's instructions.

2.7.4 Histology and Immunofluorescence

Immediately after euthanasia, the soleus muscle was dusted in talc, frozen in liquid nitrogen, and stored frozen at −80°C. Afterwards, glass slides (26 × 76 mm) were prepared by sectioning the muscles (6 μm) using a cryostat (Leica CM 1850 UV) at −25°C. The sections were stained by Hematoxilin-Eosin (H&E, MERCK, Darmstadt, Germany) to identify any morphological alterations in tissue through a light microscope.

Immunofluorescence was applied to quantify NRF-1 and PGC-1α. The slides with frozen sections were incubated in a mix of primary anti-mouse monoclonal antibodies for NRF-1 (dilution 1:500; Santa Cruz Biotechnology, INC.; Dallas, Texas, United States) or PGC-1α (dilution 1:50; Santa Cruz Biotechnology, INC.; Dallas, Texas, United States), conjugated with anti-rabbit laminin (dilution 1:200; Abcam; Ab11575; Cambridge, United kingdom) diluted in 1% BSA (Bovine Serum Albumin—Sigma Aldrich Chemical Corporation, St Louis, MO, United States) for 45 min at 37°C. Then, the slides were washed (3 cycles of 5 min) in PBS solution and incubated in

a mix of secondary antibodies: Alexa 488 IgG^1 to mark NRF-1 in green color (dilution 1:1000; Jackson ImmunoResearch, Laboratories, INC.; West Grove, PA, United States) or Alexa Fluor 647 IgG_{2a} to mark PGC-1α in red color (dilution 1:1000; Santa Cruz Biotechnology, INC.; Dallas, Texas, United States), in combination with Alexa Fluor 647 IgG (dilution 1:200; Invitrogen; Carlsbad, California, United States) to mark laminin with a red color or Alexa Fluor 488 IgG to mark laminin with a green color (dilution 1:200; Invitrogen; Carlsbad, California, United States) for 35 min at 37°C. The sections were washed (3 cycles of 5 min) in PBS solution and mounted with FluoroQuest™ Mounting Medium (AAT Bioquest®, INC., Sunnyvale, CA, United States).

The slides were analyzed with ImageXpress® Micro (Molecular Devices; San José, CA, United States) using an objective lens with magnification of 20× and specific filters for NRF-1 (FITC—1,200 ms exposure), PGC-1α (Cy5—2000 ms exposure), and laminin (FITC and Cy5—100 ms exposure). The integrated density of the fluorescence intensity of NRF-1 and PGC-1α was quantified in five distinct and random fields (height: 220 and width: 220) by ImageJ 1.52a software (National Institutes of Health, United States), followed by an individual analysis of the images. The mean value of the proteins in each sample was calculated and plotted in a graph.

2.8 Statistical Analysis

A priori power analysis was determined by G*Power 3.1.9.4 software, and it was calculated that a sample size of 40 (10 rats per group) would be required using a two-way ANOVA test at the 5% level of significance with power around 0.77, assuming an effect size of 0.5. The data were presented as a mean ± standard error of the mean. Normality and homogeneity were verified with the Shapiro-Wilk and Levene tests, respectively. When appropriate, outliers were excluded. Time to exhaustion was analyzed through the *t*-test for independent samples comparing exercised animals treated with melatonin (MEXG) versus exercised animals treated with vehicle (EXG). One-way analysis of variance (ANOVA) was used to compare the four groups with regard to the variables obtained from the graded exercise test (iLAn and lactacidemia at this intensity). Two-way ANOVA was applied to analyze other parameters regarding the effects of melatonin (melatonin vs. vehicle) and exercise (exercised vs. non-exercised). When appropriate, the Newman-Keuls post hoc test was used. A significance level of 5% and Statistica 7.0 (StatSoft, Inc.; Tulsa, OK, United States) were used for all analyses. Effect size analysis (ES) and confidence interval (CI) were used as complementary tests. The thresholds for small, moderate, and large effects were 0.20, 0.50, and 0.80, respectively. ES was determined according to Cohen (1988).

3 RESULTS

With regard to the variables obtained from the graded exercise test (GXT), no considerable differences were found among the groups in relation to iLAn (CG: 5.41 ± 0.29 (CI = 4.71–6.11), EXG: 5.32 ± 0.17 (CI = 4.92–5.73), MG: 5.51 ± 0.23 (CI = 4.90–6.13), and MEXG: 5.46 ± 0.17 (CI = 5.06–5.85) %BM; $F = 0.13$, $p = 0.94$) and lactacidemia at iLAn (CG: 4.08 ± 0.29 (CI = 3.26–4.89), EXG: 3.93 ± 0.41 (CI = 2.99–4.88), MG: 3.56 ± 0.41 (CI = 2.59–4.52), and MEXG: 4.02 ± 0.22 (CI = 3.52–4.53) mM; $F = 0.41$, $p = 0.74$). Regarding *t*lim, the animals treated with melatonin (MEXG: 105.31 ± 22.89 min; CI = 60.44–150.19) swam 120.3% more than animals treated with vehicle (EXG: 47.81 ± 10.38 min; CI = 27.46–68.15; $p < 0.01$, ES: 1.17).

Exercise and melatonin increased PGC-1α ($F = 64.64$, $p < 0.01$ and $F = 12.00$, $p < 0.01$) and NRF-1 ($F = 39.81$, $p < 0.01$ and $F = 4.20$, $p < 0.05$) (**Figure 2**). Large effects on PGC-1α were obtained when comparing CG vs. EXG ($p < 0.01$, ES: 2.84; EXG > CG), EXG vs. MG ($p < 0.01$, ES: 1.64; EXG > MG), CG vs. MEXG ($p < 0.01$, ES: 3.69; MEXG > CT), EXG vs. MEXG ($p < 0.01$, ES: 2.00; MEXG > EXG), and MG vs. MEXG ($p < 0.01$, ES: 2.64; MEXG > MG). Mean ± SEM and confidence interval values on PGC-1α for CG (8,811.76 ± 444.63; CI = 7,805.92–9,917.60), EXG (11,824.22 ± 238.15; CI = 11,275.03–12,373.42), MG (9,809.93 ± 582.68; CI = 8,466.26–11,153.60), and MEXG (13,877.66 ± 423.28; CI = 12,920.12–14,835.20). Likewise, large effects on NRF-1 were found when comparing CG vs. EXG ($p < 0.01$, ES: 2.84; EXG > CG), CG vs. MEXG ($p < 0.01$, ES: 3.69; MEXG > CG), and MG vs. MEXG ($p < 0.01$, ES: 1.87; MEXG > MG). Mean ± SEM and confidence interval on NRF-1 for CG (9,341.66 ± 389.76; CI = 8,459.94–10,223.38), EXG (13,130.80 ± 694.19; CI = 11,529.98–14,731.62), MG (10,711.16 ± 669.48; CI = 9,167.32–12,254.99), and MEXG (14,089.66 ± 508.42; CI = 12,939.53–15,239.79).

As to glycogen, exercise increased its content in red gastrocnemius ($F = 13.32$, $p < 0.01$) but decreased in liver ($F = 37.70$, $p < 0.01$), while no difference was observed in gluteus maximus and white gastrocnemius ($F = 0.35$, $p = 0.55$ and $F = 0.56$, $p = 0.45$). Furthermore, melatonin increased the glycogen content in gluteus maximus ($F = 5.71$, $p = 0.02$), but did not promote any difference in liver ($F = 3.59$, $p = 0.06$), red and white gastrocnemius ($F = 0.55$, $p = 0.46$ and $F < 0.01$, $p = 0.92$, respectively) (**Figures 3A–D**). Large effects were observed on glycogen content in liver (CG vs. EXG ($p = 0.01$, ES: 1.36; CG > EXG), CG vs. MG ($p = 0.01$, ES: 1.10; MG > CG), EXG vs. MG ($p < 0.01$, ES: 2.61; MG > EXG), CG vs. MEXG ($p < 0.01$, ES: 1.41; CG > MEXG), and MG vs. MEXG ($p < 0.01$, ES: 2.73; MG > MEXG)), gluteus maximus (CG vs. MG ($p = 0.04$, ES: 2.13; MG > CG)) and red gastrocnemius (CG vs. MEXG ($p = 0.01$, ES: 1.71; MEXG > CG) and MG vs. MEXG ($p < 0.01$, ES: 1.83; MEXG > MG)). Mean ± SEM and confidence interval on glycogen content for liver: CG (1.85 ± 0.23; CI = 1.31–2.39), EXG (0.92 ± 0.19; CI = 0.48–1.36), MG (2.65 ± 0.23; CI = 2.11–3.20), and MEXG (0.94 ± 0.18; CI = 0.52–1.36); gluteus maximus: CG (0.49 ± 0.01; CI = 0.45–0.54), EXG (0.54 ± 0.06; CI = 0.39–0.69), MG (0.68 ± 0.03; CI = 0.59–0.77), and MEXG (0.58 ± 0.01; CI = 0.53–0.62); red gastrocnemius: CG (0.54 ± 0.03; CI = 0.46–0.63), EXG (0.63 ± 0.04; CI = 0.52–0.74), MG (0.51 ± 0.04; CI = 0.39–0.62), and MEXG (0.73 ± 0.03; CI = 0.65–0.81); and white gastrocnemius: CG (0.61 ± 0.02; CI = 0.55–0.68), EXG (0.65 ± 0.05; CI = 0.52–0.78), MG (0.62 ± 0.02; CI = 0.56–0.69), and MEXG (0.65 ± 0.05; CI = 0.53–0.77).

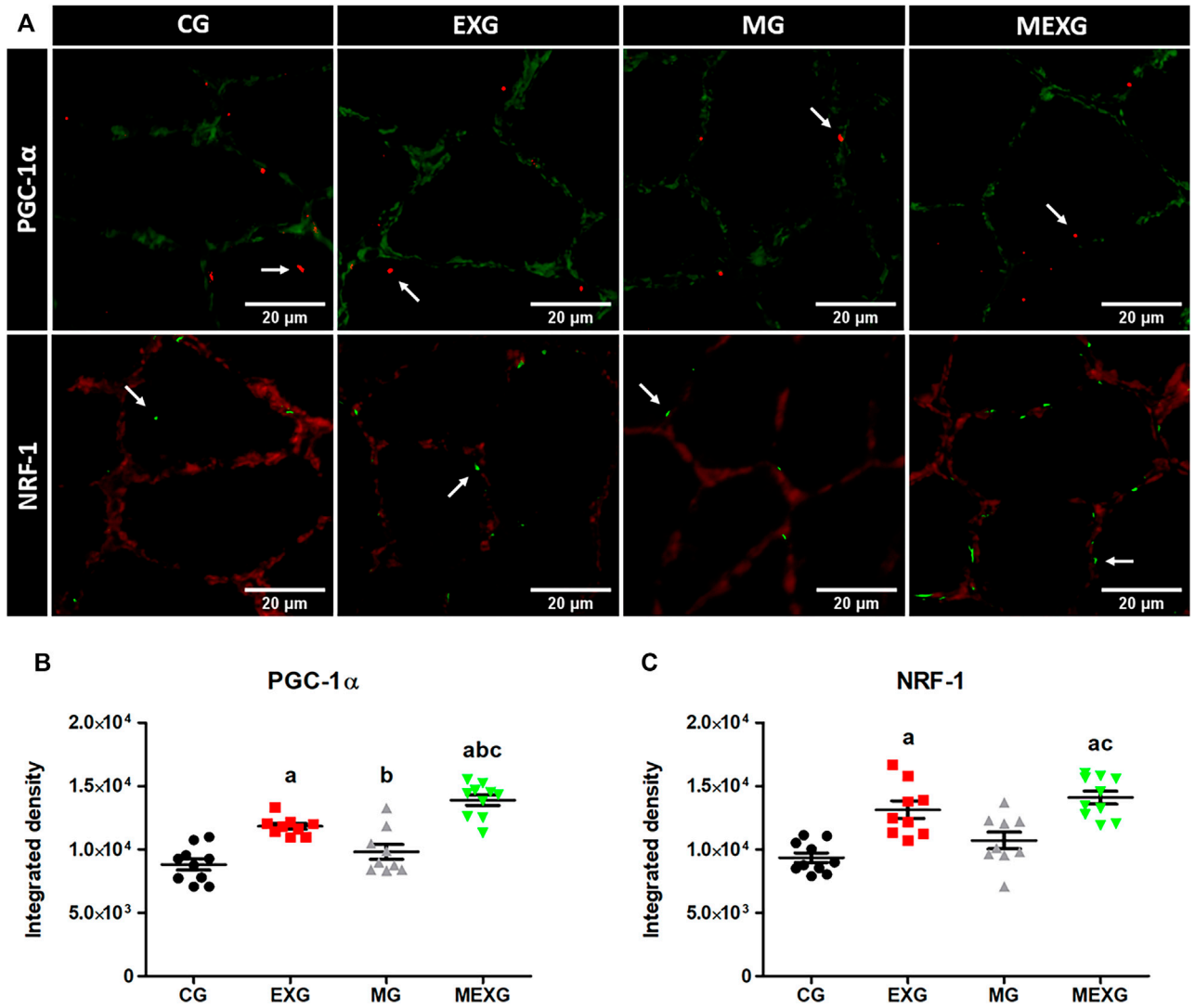

FIGURE 2 | NRF-1 and PGC-1α in skeletal muscles. Representative samples of laminin (green) with PGC-1α (red) in the soleus skeletal muscle with immunofluorescence (height: 350 and width: 350) **(A)**. Representative samples of laminin (red) with NRF-1 (green) in the soleus skeletal muscle **(A)** with immunofluorescence in the control (CG), rats treated with melatonin (MG), rats submitted to exercise (EXG), and rats treated with melatonin and submitted to exercise (MEXG). The white arrows indicate NRF-1 and PGC-1α in the soleus skeletal muscle. The graphs represent the means and standard errors of NRF-1 **(B)** and PGC-1α **(C)**. [a] $p < 0.05$ with respect to CG; [b] $p < 0.05$ with respect to EXG; [c] $p < 0.05$ with respect to MG for the same parameter. For illustration, an objective lens = 20x was used; bars = 20 μm.

In relation to triglyceride content, exercise did not cause any change in skeletal muscles, such as gluteus maximus ($F = 2.51$, $p = 0.12$), red and white gastrocnemius ($F = 2.23$, $p = 0.14$ and $F = 0.18$, $p = 0.66$, respectively). On the other hand, melatonin decreased the triglyceride content in gluteus maximus ($F = 6.66$, $p = 0.01$) red and white gastrocnemius ($F = 4.51$, $p = 0.04$ and $F = 6.02$, $p = 0.02$) (**Figures 3E–G**). Large effects on the triglyceride content in gluteus maximus were noted when comparing CG vs. EXG ($p < 0.01$, ES: 1.63; EXG > CG), EXG vs. MG ($p < 0.01$, ES: 2.22; EXG > MG), and EXG vs. MEXG ($p < 0.01$, ES: 1.84; EXG > MEXG). Mean ± SEM and confidence interval on triglyceride content for gluteus maximus: CG (1.16 ± 0.04; CI = 1.07–1.25), EXG (1.36 ± 0.03; CI = 1.27–1.45), MG (1.19 ± 0.01; CI = 1.14–1.23), and MEXG (1.12 ± 0.04; CI = 1.01–1.23); red gastrocnemius: CG (1.42 ± 0.05; CI = 1.29–1.56), EXG (1.35 ± 0.07; CI = 1.18–1.53), MG (1.32 ± 0.04; CI = 1.21–1.43), and MEXG (1.22 ± 0.02; CI = 1.15–1.28); and white gastrocnemius: CG (1.19 ± 0.07; CI = 1.03–1.35), EXG (1.25 ± 0.02; CI = 1.20–1.29), MG (1.10 ± 0.03; CI = 1.03–1.18), and MEXG (1.09 ± 0.04; CI = 0.98–1.20).

4 DISCUSSION

Among the main findings of this study, we can highlight the ability of melatonin to potentiate exercise-mediated increases in

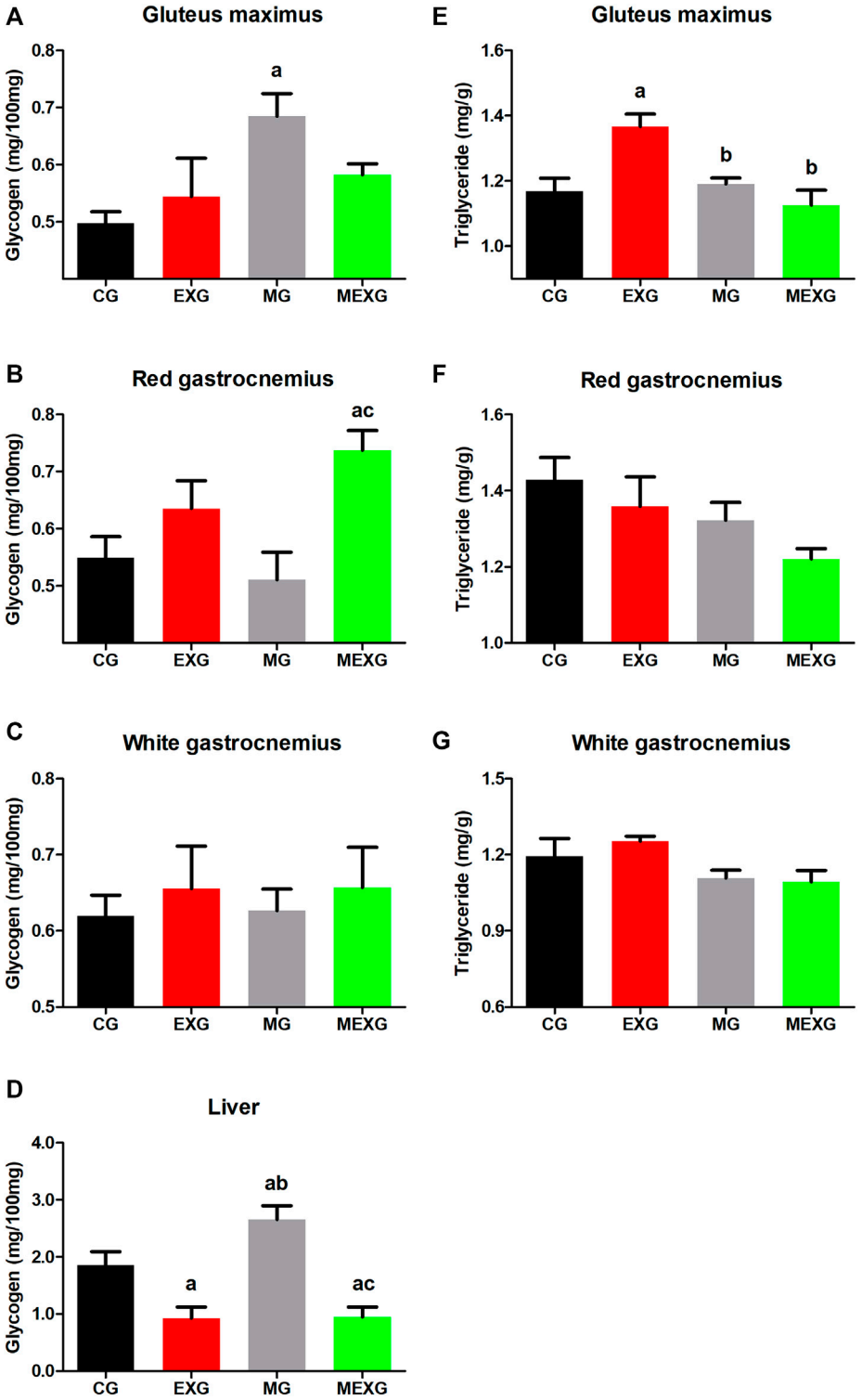

FIGURE 3 | Glycogen content in skeletal muscles and liver, and triglyceride content in skeletal muscles. The graphs represent the means and standard errors of the glycogen content in the gluteus maximus **(A)**, red **(B)** and white **(C)** gastrocnemius and liver **(D)**, and triglyceride content in gluteus maximus **(E)**, red **(F)** and white **(G)** gastrocnemius in control rats (CG), rats treated with melatonin (MG), rats submitted to exercise (EXG), and rats treated with melatonin and submitted to exercise (MEXG). [a] $p < 0.05$ with respect to CG; [b] $p < 0.05$ with respect to EXG; [c] $p < 0.05$ with respect to MG for the same parameter. mg, milligrams; g, grams; min, minutes.

PGC-1α, to reduce muscle triglyceride content and to increase glycogen content 3 h after exhaustive exercise session, possibly favoring cellular environment for future efforts, thus confirming our initial hypothesis. This is the first study to analyze the acute effect of melatonin administration on PGC-α and NRF-1 and its influence on the replenishment of glycogen in rats submitted to an individualized exhaustive exercise session with intensity corresponding to the anaerobic lactacidemic threshold.

To evaluate the beneficial effect of melatonin and exercise on the mitochondrial biogenesis, we quantified PGC-1α, a transcriptional coactivator that functions as a master regulator of the mitochondrial biogenesis (Booth et al., 2015). With regard to the effect of melatonin, PGC-1α and NRF-1 of the animals treated with melatonin showed an increase compared to those treated with vehicle ($F = 12.00$, $p < 0.01$ and $F = 4.20$, $p < 0.05$, respectively). These effects possibly occurred via melatonin receptors that were found to be present in the skeletal muscle membrane [Ha et al., 2006; (BioGPS, 2021) (http://ds.biogps.org/?dataset=GSE952&gene=114211)]. The literature postulates that melatonin acts through the activation of CAMKII, inducing CREB phosphorylation, and consequently increasing the expression of PGC-1α in the skeletal muscle (Teodoro et al., 2014). Such increase leads to the activation of key genes involved in the mitochondrial biogenesis, such as NRF-1 (Jung and Kim, 2014; Islam et al., 2020). Additionally, it seems that the activation pathway of PGC-1α is tissue-dependent. In heart tissue, melatonin can act via AMPK-PGC-1α to improve the mitochondrial biogenesis (Yu et al., 2017; Qi and Wang, 2020). Therefore, even though a positive effect of melatonin was observed a few hours after acute administration, further studies are still necessary to better understand the pathways involved in the activation of PGC-1α in the skeletal muscle of animals treated with melatonin.

Regarding the effect of exercise, the PGC-1α and NRF-1 of exercised animals showed higher expression in relation to non-exercised animals ($F = 64.64$, $p < 0.01$ and $F = 39.81$, $p < 0.01$, respectively). It is well known that exercise is one of the main stimuli for PGC-1α activation. Thus, according to the literature a single bout of exercise can activate calcium/calmodulin-dependent protein kinase (CaMK), p38 mitogen-activated protein kinase (p38 MAPK), cyclic adenosine monophosphate (cAMP), and phosphorylate AMP activated protein kinase (AMPK), which are the molecular signals responsible for the increase in PGC-1α expression (Bonen, 2009; Lira et al., 2010; Perry and Hawley, 2018; Memme et al., 2021). Our results are in accordance with the literature and demonstrate that a single bout of exercise increases the expression and/or content of PGC-1α (Wright et al., 2007; Ikeda et al., 2008; Seebacher and Glanville, 2010; Fujimoto et al., 2011; Shute et al., 2018) and NRF-1 (Murakami et al., 1998; Seebacher and Glanville, 2010; Daussin et al., 2012). More importantly, our main finding is that melatonin potentiates the up-regulation of PGC-1α expression. Curiously, the PGC-1α up-regulation mediated by melatonin was found only in exercised rats (but not in non-exercised animals). These observations suggest that melatonin effects are pronounced during challenging situations. This is in congruence with the findings from previous studies conducted by our group, who reported the effect of melatonin only in the presence of a stressful stimulus, such exhaustive exercise (Beck et al., 2015A). Furthermore, other authors demonstrated interesting effects of melatonin on *in vitro* palmitic acid-induced insulin resistance model or *in vivo* pinealectomized rats, evidencing the increase in the PGC-1α expression in both situations (Teodoro et al., 2014). In line with this rationale, melatonin (in the absence of a stressful stimulus of exercise) did not cause any change in PGC-1α and NRF-1 when compared to non-exercised animals treated with vehicle (MG vs. CG; $p > 0.05$).

Concerning exercise performance, compelling evidence has shown a significant lower performance in isometric and dynamic muscle endurance, assessed by muscle grip strength test and graded exhaustive running treadmill exercise test in skeletal muscle-specific PGC-1α knockout (PGC-1α MKO) (Handschin et al., 2007) or whole-body PGC-1α knockout mice (PGC-1α KO) (Leone et al., 2005) when compared to control animals. According to Leone et al. (2005), the exercise capacity was partly reduced due to abnormalities in the mitochondrial structure of the skeletal muscle and the function of PGC-1α KO in animals with lower maximal oxygen consumption (VO_{2max}) and fatigue resistance index than control mice ($p < 0.05$).

In an opposite scenario of low PGC-1α, there is evidence showing that animals with overexpressed skeletal muscle-specific PGC-1α (PGC-1α MKC) reached a longer distance, obtained a higher oxygen uptake (VO_2) (Wong et al., 2015), and peak oxygen consumption (VO_{2peak}) (Tadaishi et al., 2011) than control animals during graded maximal exercise test ($p < 0.05$). Therefore, when analyzing our results, we believe that the increase in exhaustion time presented by animals treated with melatonin (MEXG) in comparison with animals treated with vehicle (EXG: $p < 0.01$, ES: 1.17) may have occurred due to two factors: 1) the ability of melatonin to potentiate the effect of muscle contraction on PGC-1α (as seen by MEXG with respect to EXG; $p = 0.002$, ES: 2.00), thus allowing greater exercise tolerance. In line with this, Wong et al. (2015) and Tadaishi et al. (2011) demonstrated a significantly positive relationship between exercise tolerance and PGC-1α. 2) According to Sanchez-Campos et al. (2001) and Mazepa et al. (2000), the animals treated with melatonin and euthanized approximately 2 h (30 min + time to exhaustion) after melatonin administration obtained a higher liver and/or muscle glycogen content than control animals ($p < 0.05$). A similar behavior was observed in our results, which confirmed the ability of melatonin to increase the glycogen content in animals euthanized approximately 4 h (30 min + time to exhaustion + 3 h) after melatonin administration (as seen by MG compared to CG for gluteus maximus and liver ($p < 0.05$; ES: 2.13 and ES: 1.10, respectively)). Thus, considering that this exercise model (*t*lim) considerably depleted the muscle glycogen content (Beck et al., 2014) and that its absence is a limiting factor for performance (Krssak et al., 2000), we believe that the increase in time to exhaustion (*t*lim) presented by MEXG in comparison with EXG ($p < 0.01$, ES: 1.17) may also have been a result of the increase in the pre-exercise glycogen content. However, there are no studies involving the acute effect of melatonin on the pre-exercise glycogen content (30 min after administration) and its subsequent use during exhaustive exercise (*t*lim). Hence, more studies must be conducted to deepen the understanding of such issue.

Concerning metabolic recovery, the mitochondrial capacity for substrate oxidation in skeletal muscle is the major determinant of performance (Halling et al., 2019), as well as the metabolic recovery

after physical exercise, through the replenishment of glycogen. Besides the role in the biogenesis and mitochondrial function, PGC-1α and NRF-1 are directly linked to the regulation of energy substrates for the skeletal muscle (Bonen, 2009), resulting in a profound increase in its capacity to use lipid substrate (Wong et al., 2015). We then believe that the increase in PGC-1α and NRF-1 led to a lower triglyceride content, as demonstrated by the animals treated with melatonin in relation to those treated with vehicle for all skeletal muscles, such as gluteus maximus ($F = 6.66$, $p = 0.01$), red and white gastrocnemius ($F = 4.51$, $p = 0.04$ and $F = 6.02$, $p = 0.02$). These results are in accordance with the literature, which demonstrated a better lipid profile for animals treated with melatonin, decreasing intramuscular fat deposition by promoting lipolysis and increasing mitochondrial function in porcine intramuscular preadipocytes (Liu et al., 2019), as well as reduced blood triglyceride concentration (Agil et al., 2011; Mendes et al., 2013; Lolei et al., 2019). Considering the reduction in the muscle triglyceride content, a higher glycogen content was expected in gluteus maximus, as demonstrated by the animals treated with melatonin compared with those treated with vehicle ($F = 5.71$, $p = 0.02$).

In relation to glycogen replenishment, it is important to note that the animals treated with melatonin (MEXG) swam 120.3% more than those treated with vehicle (EXG; $p < 0.05$, ES: 1.17), which made us expect a lower content of glycogen, as suggested by the literature (Matsui et al., 2011; Beck et al., 2014). However, when analyzing our results, the animals treated with melatonin and submitted to exercise (MEXG) were statistically equal to those treated with vehicle and submitted to exercised (EXG) in terms of glycogen content in the liver ($p > 0.05$) and the skeletal muscles (gluteus maximus, red and white gastrocnemius ($p > 0.05$)), even though a difference in *t*lim appeared between the groups (MEXG > EXG; $p < 0.05$). Moreover, in the presence of melatonin, there was an overcompensation of the glycogen content in the red gastrocnemius of exercised animals (MEXG; $p = 0.01$, ES: 1.71). However, this did not occur in the absence of melatonin (EXG; $p > 0.05$) in comparison with the control animals (CG). Therefore, we can consider that melatonin accelerates the replenishment of energy substrates, which may have facilitated the increase in glycogen stores.

In this scenario, a rapid glycogen repletion following a bout of exhausting intense exercise is an important response to prepare the muscle for subsequent bouts of activity (Wende et al., 2007), specially for sports with repeated bouts of exercise at the same day or in the following day. Thereby, melatonin seems to optimize the response to exercise since training adaptations reflect the accumulation of beneficial physiological functions produced from single bouts of exercise (Park et al., 2021). Despite the positive results of this study, there are still some limitations that must be addressed: 1) We only focused on the master regulator of the mitochondrial biogenesis and its relationship with NRF-1; however, evaluating other proteins involved in the mitochondrial biogenesis process as well as the PGC-1α downstream signals could provide additional information on the mechanism of action of melatonin; 2) we chose the dosage of 10 mg/kg due to the effects of melatonin on time to exhaustion (*t*lim), as previously reported by our research group (Beck et al., 2015A; Beck et al., 2016; Faria et al., 2021); nonetheless, another concentration of melatonin should be tested to achieve a similar effect with lower dosage. Therefore, our findings make it clear that future studies must be conducted in order to deepen the understanding of the importance of melatonin from a physiological point of view.

In summary, the current study highlighted the role of melatonin in the increase of exercise tolerance, exercise-mediated PGC-1α and muscle glycogen after exhaustive prolonged exercise, as well as in the decrease of muscle triglyceride content, thus providing a better cellular metabolism environment for future efforts and virtually improving adaptive responses to training.

5 FUTURE PERSPECTIVES

If confirmed in humans, the outcomes of this study could be useful for athletes who must quickly return to their training or competitive activities; For sure, further studies are needed to elucidate whether such effects occur similarly in humans. In addition, the administration of melatonin in the context of training recovery should be more explored and expanded to other health areas. In this line, melatonin could be useful in treating/avoiding overtraining, a condition in which energy stores as glycogen are chronically low (Fry et al., 1991; Smith, 2000).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Ethics Committee on the Animal Use of the Federal University of São Carlos—UFSCar (São Paulo, Brazil) under protocol no. 9144181218.

AUTHOR CONTRIBUTIONS

WB and VF contributed to the conception and design of the research. VF conducted the experiments and wrote the first draft of the manuscript. WB, PS, FM-G, AZ, and VF analyzed data, reviewed and approved the manuscript.

FUNDING

This study was partly financed by the São Paulo Research Foundation (FAPESP, Process No. 2019/08148-3) and the Coordination for the Improvement of Higher Education Personnel (CAPES, Process No. 88882.426921/2019-01; finance code 001).

ACKNOWLEDGMENTS

We are grateful to the Laboratory of Applied Sport Physiology (UNICAMP, Limeira/São Paulo) for their provision of the tanks for the swimming training, the Immunohistochemistry

Laboratory (UFSCar, São Carlos/São Paulo) for the use of the Leica CM 1850 UV Cryostat (São Paulo Research Foundation; Process No. 2013/21621-3), the Laboratory of Biochemistry and Molecular Biology (UFSCar, São Carlos/São Paulo) for the use of ImageXpress and SpectraMax i3 (São Paulo Research Foundation; Process No. 2014/50256-4), and the Muscle Physiology and Biophysics Laboratory (Federal University of São Carlos, São Paulo) for the immunofluorescence procedures.

REFERENCES

Acuña-Castroviejo, D., Escames, G., Venegas, C., Díaz-Casado, M. E., Lima-Cabello, E., López, L. C., et al. (2014). Extrapineal Melatonin: Sources, Regulation, and Potential Functions. *Cell. Mol. Life Sci.* 71 (16), 2997–3025. doi:10.1007/s00018-014-1579-2

Agil, A., Navarro-Alarcón, M., Ruiz, R., Abuhamadah, S., El-Mir, M.-Y., and Vázquez, G. F. (2010). Beneficial Effects of Melatonin on Obesity and Lipid Profile in Young Zucker Diabetic Fatty Rats. *J. Pineal Res.* 50 (2), no. doi:10.1111/j.1600-079X.2010.00830.x

Amaral, F. G. D., Andrade-Silva, J., Kuwabara, W. M. T., and Cipolla-Neto, J. (2019). New Insights into the Function of Melatonin and its Role in Metabolic Disturbances. *Expert Rev. Endocrinol. Metab.* 14 (4), 293–300. doi:10.1080/17446651.2019.1631158

American Veterinary Medical Association (2013). *AVMA Guidelines on Euthanasia*. Illinois: AVMA, Schaumber.

Animal Research Review Panel (2007). *ARRP Guideline 20: Guidelines for the Housing of Rats in Scientific Institutions*. Orange, NSW: Animal Welfare Branch, NSW Department of Primary Industries.

APS (2006). *Resource Book for the Design of Animal Exercise Protocols*, 137. Rockville, MD: American Physiological Society

Beck, W. F., De, G. G., Menezes Scariot, P. P., Masselli dos Reis, I. G., and Gobatto, C. A. (2014). Time to Exhaustion at Anaerobic Threshold in Swimming Rats: Metabolic Investigation. *Bll* 115 (10), 617–621. doi:10.4149/bll_2014_119

Beck, W., and Gobatto, C. (2013). Effects of Maximum Intensity Aerobic Swimming Exercise until Exhaustion at Different Times of Day on the Hematological Parameters in Rats. *Acta Physiol. Hungarica* 100 (4), 427–434. doi:10.1556/APhysiol.100.2013.013

Beck, W. R., Botezelli, J. D., Pauli, J. R., Ropelle, E. R., and Gobatto, C. A. (2015). Melatonin Has an Ergogenic Effect but Does Not Prevent Inflammation and Damage in Exhaustive Exercise. *Sci. Rep.* 5, 1–10. doi:10.1038/srep18065

Beck, W. R., Campesan, Y. S., and Gobatto, C. A. (2015). Validity and Reliability of Incremental Test to Determine the Anaerobic Threshold in Swimming Rats. *Int. J. Appl. Exerc. Physiol.* 4 (1), 25–33.

Beck, W., Scariot, P., and Gobatto, C. (2016). Melatonin Is an Ergogenic Aid for Exhaustive Aerobic Exercise Only during the Wakefulness Period. *Int. J. Sports Med.* 37 (1), 71–76. doi:10.1055/s-0035-1559698

BioGPS (2021). Spotlight on the Sheep Gene Expression Atlas. AvaliableAt: http://ds.biogps.org/?dataset=GSE952&gene=114211 (Accessed October 1st, 2021).

Bonen, A. (2009). PGC-1α-induced Improvements in Skeletal Muscle Metabolism and Insulin sensitivityThis Paper Is One of a Selection of Papers Published in This Special Issue, Entitled 14th International Biochemistry of Exercise Conference - Muscles as Molecular and Metabolic Machines, and Has Undergone the Journal's Usual Peer Review Process. *Appl. Physiol. Nutr. Metab.* 34 (3), 307–314. doi:10.1139/H09-008

Booth, F. W., Ruegsegger, G. N., Toedebusch, R. G., and Yan, Z. (2015). Endurance Exercise and the Regulation of Skeletal Muscle Metabolism. *Prog. Mol. Biol. Transl. Sci.* 135, 129–151. doi:10.1016/bs.pmbts.2015.07.016

Brzezinski, A., Rai, S., Purohit, A., and Pandi-Perumal, S. R. (2021). Melatonin, Clock Genes, and Mammalian Reproduction: What Is the Link? *Ijms* 2224, 13240. doi:10.3390/ijms222413240

Burke, L. M., and Hawley, J. A. (2018). Swifter, Higher, Stronger: What's on the Menu? *Science* 362, 781–787. doi:10.1126/science.aau2093

Castelhano-Carlos, M. J., and Baumans, V. (2009). The Impact of Light, Noise, Cage Cleaning and In-House Transport on Welfare and Stress of Laboratory Rats. *Lab. Anim.* 43 (4), 311–327. doi:10.1258/la.2009.0080098

Chitimus, D. M., Popescu, M. R., Voiculescu, S. E., Panaitescu, A. M., Pavel, B., Zagrean, L., et al. (2020). Melatonin's Impact on Antioxidative and Anti-inflammatory Reprogramming in Homeostasis and Disease. *Biomolecules* 10, 1211. doi:10.3390/biom10091211

Cohen, D. (1988). *Statistical Power Analysis for the Behavioral Sciences*. Hillsdale: Lawrence Erlbaum Associates.

Daussin, F. N., Rasseneur, L., Bouitbir, J., Charles, A.-L., Dufour, S. P., Geny, B., et al. (2012). Different Timing of Changes in Mitochondrial Functions Following Endurance Training. *Med. Sci. Sports Exerc.* 44 (2), 217–224. doi:10.1249/MSS.0b013e31822b0bd4

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Anal. Chem.* 28, 350–356. doi:10.1021/ac60111a017

Faria, V. S., Pejon, T. M. M., Gobatto, C. A., de Araújo, G. G., Cornachione, A. S., and Beck, W. R. (2021). Acute Melatonin Administration Improves Exercise Tolerance and the Metabolic Recovery after Exhaustive Effort. *Sci. Rep.* 11 (1), 19228. doi:10.1038/s41598-021-97364-7

Fry, R. W., Morton, A. R., and Keast, D. (1991). Overtraining in Athletes. *Sports Med.* 12 (1), 32–65. doi:10.2165/00007256-199112010-00004

Fujimoto, E., Yamaguchi, W., Terada, S., Higuchi, M., and Tabata, I. (2011). Change in PGC-1α Expression in Rat Skeletal Muscle after Low-Intensity Prolonged Swimming Exercise. *J. Physiol. Anthropol.* 30 (1), 23–27. doi:10.2114/jpa2.30.23

Galano, A., Tan, D.-X., and Reiter, R. (2018). Melatonin: a Versatile Protector against Oxidative DNA Damage. *Molecules* 23 (3), 530. doi:10.3390/molecules23030530

Ha, E., Yim, S.-V., Chung, J.-H., Yoon, K.-S., Kang, I., Cho, Y. H., et al. (2006). Melatonin Stimulates Glucose Transport via Insulin Receptor Substrate-1/phosphatidylinositol 3-kinase Pathway in C2C12 Murine Skeletal Muscle Cells. *J. Pineal Res.* 41 (1), 67–72. doi:10.1111/j.1600-079X.2006.00334.x

Halling, J. F., Jessen, H., Nøhr-Meldgaard, J., Buch, B. T., Christensen, N. M., Gudiksen, A., et al. (2019). PGC-1α Regulates Mitochondrial Properties beyond Biogenesis with Aging and Exercise Training. *Am. J. Physiology-Endocrinology Metab.* 317 (3), E513–E525. doi:10.1152/ajpendo.00059.2019

Handschin, C., Chin, S., Li, P., Liu, F., Maratos-Flier, E., LeBrasseur, N. K., et al. (2007). Skeletal Muscle Fiber-type Switching, Exercise Intolerance, and Myopathy in PGC-1α Muscle-specific Knock-Out Animals. *J. Biol. Chem.* 282 (41), 30014–30021. doi:10.1074/jbc.M704817200

Hardeland, R. (2022). Redox Biology of Melatonin: Discriminating between Circadian and Noncircadian Functions. *Antioxid. Redox Signaling*. doi:10.1089/ars.2021.0275

Hargreaves, M., and Spriet, L. L. (2020). Skeletal Muscle Energy Metabolism during Exercise. *Nat. Metab.* 2 (9), 817–828. doi:10.1038/s42255-020-0251-4

Ikeda, S.-i., Kizaki, T., Haga, S., Ohno, H., and Takemasa, T. (2008). Acute Exercise Induces Biphasic Increase in Respiratory mRNA in Skeletal Muscle. *Biochem. Biophysical Res. Commun.* 368 (2), 323–328. doi:10.1016/j.bbrc.2008.01.095

Islam, H., Edgett, B. A., and Gurd, B. J. (2018). Coordination of Mitochondrial Biogenesis by PGC-1α in Human Skeletal Muscle: A Re-evaluation. *Metabolism* 79, 42–51. doi:10.1016/j.metabol.2017.11.001

Islam, H., Hood, D. A., and Gurd, B. J. (2020). Looking beyond PGC-1α: Emerging Regulators of Exercise-Induced Skeletal Muscle Mitochondrial Biogenesis and Their Activation by Dietary Compounds. *Appl. Physiol. Nutr. Metab.* 45 (1), 11–23. doi:10.1139/apnm-2019-0069

Jung, S., and Kim, K. (2014). Exercise-induced PGC-1α Transcriptional Factors in Skeletal Muscle. *Integr. Med. Res.* 3 (4), 155–160. doi:10.1016/j.imr.2014.09.004

Krssak, M., Petersen, K. F., Price, T., Laurent, D., Rothman, D. L., Roden, M., et al. (2000). Intramuscular Glycogen and Intramyocellular Lipid Utilization during Prolonged Exercise and Recovery in Man: a 13C and 1H Nuclear Magnetic Resonance Spectroscopy Study. *J. Clin. Endocrinol. Metab.* 85 (2), 748–754. doi:10.1210/jcem.85.2.635410.1210/jc.85.2.748

Kruk, J., Aboul-Enein, B. H., and Duchnik, E. (2021). Exercise-induced Oxidative Stress and Melatonin Supplementation: Current Evidence. *J. Physiol. Sci.* 71 (1), 1–19. doi:10.1186/s12576-021-00812-2

Leckey, J. J., Burke, L. M., Morton, J. P., and Hawley, J. A. (2016). Altering Fatty Acid Availability Does Not Impair Prolonged, Continuous Running to Fatigue:

Evidence for Carbohydrate Dependence. *J. Appl. Physiol.* 120, 107–113. doi:10.1152/japplphysiol.00855.2015

Leone, T. C., Lehman, J. J., Finck, B. N., Schaeffer, P. J., Wende, A. R., Boudina, S., et al. (2005). PGC-1α Deficiency Causes Multi-System Energy Metabolic Derangements: Muscle Dysfunction, Abnormal Weight Control and Hepatic Steatosis. *Plos Biol.* 3 (4), e101. doi:10.1371/journal.pbio.0030101

Lima, A. A. D., Gobatto, G. A., Messias, L. H. D., Scariot, P. P. M., Forte, L. D. M., Santin, J. D., et al. (2017). Two Water Environment Adaptation Models Enhance Motor Behavior and Improve the success of the Lactate Minimum Test in Swimming Rats. *Motriz* 23, e101607. doi:10.1590/s1980-6574201700si0009

Lira, V. A., Benton, C. R., Yan, Z., and Bonen, A. (2010). PGC-1α Regulation by Exercise Training and its Influences on Muscle Function and Insulin Sensitivity. *Am. J. Physiology-Endocrinology Metab.* 299 (2), E145–E161. doi:10.1152/ajpendo.00755.2009

Liu, K., Yu, W., Wei, W., Zhang, X., Tian, Y., Sherif, M., et al. (2019). Melatonin Reduces Intramuscular Fat Deposition by Promoting Lipolysis and Increasing Mitochondrial Function. *J. Lipid Res.* 60 (4), 767–782. doi:10.1194/jlr.M087619

Loloei, S., Sepidarkish, M., Heydarian, A., Tahvilian, N., Khazdouz, M., Heshmati, J., et al. (2019). The Effect of Melatonin Supplementation on Lipid Profile and Anthropometric Indices: A Systematic Review and Meta-Analysis of Clinical Trials. *Diabetes Metab. Syndr. Clin. Res. Rev.* 13 (3), 1901–1910. doi:10.1016/j.dsx.2019.04.043

Manchester, L. C., Coto-Montes, A., Boga, J. A., Andersen, L. P. H., Zhou, Z., Galano, A., et al. (2015). Melatonin: an Ancient Molecule that Makes Oxygen Metabolically Tolerable. *J. Pineal Res.* 59 (4), 403–419. doi:10.1111/jpi.12267

Matsui, T., Soya, S., Okamoto, M., Ichitani, Y., Kawanaka, K., and Soya, H. (2011). Brain Glycogen Decreases during Prolonged Exercise. *J. Physiol.* 589, 3383–3393. doi:10.1113/jphysiol.2010.20357010.1113/jphysiol.2011.203570

Matsumoto, I., Araki, H., Tsuda, K., Odajima, H., Nishima, S., Higaki, Y., et al. (1999). Effects of Swimming Training on Aerobic Capacity and Exercise Induced Bronchoconstriction in Children with Bronchial Asthma. *Thorax* 54 (3), 196–201. doi:10.1136/thx.54.3.196

Mauriz, J. L., Collado, P. S., Veneroso, C., Reiter, R. J., and González-Gallego, J. (2013). A Review of the Molecular Aspects of Melatonin's Anti-inflammatory Actions: Recent Insights and New Perspectives. *J. Pineal Res.* 54 (1), 1–14. doi:10.1111/j.1600-079X.2012.01014.x

Mazepa, R. C., Cuevas, M. J., Collado, P. S., and González-Gallego, J. (2000). Melatonin Increases Muscle and Liver Glycogen Content in Nonexercised and Exercised Rats. *Life Sci.* 66 (2), 153–160. doi:10.1016/s0024-3205(99)00573-1

Memme, J. M., Erlich, A. T., Phukan, G., and Hood, D. A. (2021). Exercise and Mitochondrial Health. *J. Physiol.* 599 (3), 803–817. doi:10.1113/JP278853

Mendes, C., Lopes, A. M. d. S., do Amaral, F. G., Peliciari-Garcia, R. A., Turati, A. d. O., Hirabara, S. M., et al. (2013). Adaptations of the Aging Animal to Exercise: Role of Daily Supplementation with Melatonin. *J. Pineal Res.* 55 (3), 229–239. doi:10.1111/jpi.12065

Murakami, T., Shimomura, Y., Yoshimura, A., Sokabe, M., and Fujitsuka, N. (1998). Induction of Nuclear Respiratory Factor-1 Expression by an Acute Bout of Exercise in Rat Muscle. *Biochim. Biophys. Acta (Bba) - Gen. Subjects* 13811, 113–122. doi:10.1016/s0304-4165(98)00018-x

Ortiz-Franco, M., Planells, E., Quintero, B., Acuña-Castroviejo, D., Rusanova, I., Escames, G., et al. (2017). Effect of Melatonin Supplementation on Antioxidant Status and DNA Damage in High Intensity Trained Athletes. *Int. J. Sports Med.* 38 (14), 1117–1125. doi:10.1055/s-0043-119881

Park, J., Kim, J., and Mikami, T. (2021). Exercise-induced Lactate Release Mediates Mitochondrial Biogenesis in the hippocampus of Mice via Monocarboxylate Transporters. *Front. Physiol.* 12, 736905. doi:10.3389/fphys.2021.736905

Perry, C. G. R., and Hawley, J. A. (2018). Molecular Basis of Exercise-Induced Skeletal Muscle Mitochondrial Biogenesis: Historical Advances, Current Knowledge, and Future Challenges. *Cold Spring Harb. Perspect. Med.* 8, 9a029686. doi:10.1101/cshperspect.a029686

Pevet, P., Challet, E., and Felder-Schmittbuhl, M.-P. (2021). Melatonin and the Circadian System: Keys for Health with a Focus on Sleep. *Handb Clin. Neurol.* 179, 331–343. doi:10.1016/B978-0-12-819975-6.00021-2

Qi, X., and Wang, J. (2020). Melatonin Improves Mitochondrial Biogenesis through the AMPK/PGC1α Pathway to Attenuate Ischemia/reperfusion-Induced Myocardial Damage. *Aging* 12 (8), 7299–7312. doi:10.18632/aging.103078

Reiter, R. J., Mayo, J. C., Tan, D.-X., Sainz, R. M., Alatorre-Jimenez, M., and Qin, L. (2016). Melatonin as an Antioxidant: under Promises but over Delivers. *J. Pineal Res.* 61 (3), 253–278. doi:10.1111/jpi.12360

Reiter, R. J., Tan, D.-X., and Fuentes-Broto, L. (2010). Melatonin: a Multitasking Molecule. *Prog. Brain Res.* 181, 127–151. doi:10.1016/S0079-6123(08)81008-4

Rodriguez, C., Mayo, J. C., Sainz, R. M., Antolin, I., Herrera, F., Martin, V., et al. (2004). Regulation of Antioxidant Enzymes: a Significant Role for Melatonin. *J. Pineal Res.* 36 (1), 1–9. doi:10.1046/j.1600-079x.2003.00092.x

Sánchez-Campos, S., Arévalo, M., Mesonero, M. J., Esteller, A., González-Gallego, J., and Collado, P. S. (2001). Effects of Melatonin on Fuel Utilization in Exercised Rats: Role of Nitric Oxide and Growth Hormone. *J. Pineal Res.* 31 (2), 159–166. doi:10.1034/j.1600-079x.2001.310210.x

Seebacher, F., and Glanville, E. J. (2010). Low Levels of Physical Activity Increase Metabolic Responsiveness to Cold in a Rat (Rattus Fuscipes). *PLoS One* 5 (9), e13022. doi:10.1371/journal.pone.0013022

Shute, R. J., Heesch, M. W., Zak, R. B., Kreiling, J. L., and Slivka, D. R. (2018). Effects of Exercise in a Cold Environment on Transcriptional Control of PGC-1α. *Am. J. Physiology-Regulatory, Integr. Comp. Physiol.* 314 (6), R850–R857. doi:10.1152/ajpregu.00425.2017

Smith, L. L. (2000). Cytokine Hypothesis of Overtraining: a Physiological Adaptation to Excessive Stress? *Med. Sci. Sports Exerc.* 32 (2), 317–331. doi:10.1097/00005768-200002000-00011

Sun, J. H., Yaga, K., Reiter, R. J., Garza, M., Manchester, L. C., Tan, D. X., et al. (1993). Reduction in Pineal N-Acetyltransferase Activity and Pineal and Serum Melatonin Levels in Rats After Their Exposure to Red Light at Night. *Neurosci Lett.* 149 (1), 56–58. doi:10.1016/0304-3940(93)90346-m

Tadaishi, M., Miura, S., Kai, Y., Kano, Y., Oishi, Y., and Ezaki, O. (2011). Skeletal Muscle-specific Expression of PGC-1α-B, an Exercise-Responsive Isoform, Increases Exercise Capacity and Peak Oxygen Uptake. *PloS one* 6 (12), e28290. doi:10.1371/journal.pone.0028290

Teodoro, B. G., Baraldi, F. G., Sampaio, I. H., Bomfim, L. H. M., Queiroz, A. L., Passos, M. A., et al. (2014). Melatonin Prevents Mitochondrial Dysfunction and Insulin Resistance in Rat Skeletal Muscle. *J. Pineal Res.* 57 (2), 155–167. doi:10.1111/jpi.12157

Wende, A. R., Schaeffer, P. J., Parker, G. J., Zechner, C., Han, D.-H., Chen, M. M., et al. (2007). A Role for the Transcriptional Coactivator PGC-1α in Muscle Refueling. *J. Biol. Chem.* 282 (50), 36642–36651. doi:10.1074/jbc.M707006200

Wong, K. E., Mikus, C. R., Slentz, D. H., Seiler, S. E., DeBalsi, K. L., Ilkayeva, O. R., et al. (2015). Muscle-Specific Overexpression of PGC-1α Does Not Augment Metabolic Improvements in Response to Exercise and Caloric Restriction. *Diabetes* 64 (5), 1532–1543. doi:10.2337/db14-0827

Wright, D. C., Han, D.-H., Garcia-Roves, P. M., Geiger, P. C., Jones, T. E., and Holloszy, J. O. (2007). Exercise-induced Mitochondrial Biogenesis Begins before the Increase in Muscle PGC-1α Expression. *J. Biol. Chem.* 282 (1), 194–199. doi:10.1074/jbc.M606116200

Yu, L., Gong, B., Duan, B., Fan, C., Zhang, J., and Li, Z. (2017). Melatonin Ameliorates Myocardial Ischemia/reperfusion Injury in Type 1 Diabetic Rats by Preserving Mitochondrial Function: Role of AMPK-PGC-1α-SIRT3 Signaling. *Sci. Rep.* 7, 1–13. doi:10.1038/srep41337

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The reviewer MS-F declared a shared affiliation with the author AZ to the handling editor at the time of review.

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frontiers | Frontiers in Physiology

ORIGINAL RESEARCH
published: 08 June 2022
doi: 10.3389/fphys.2022.833580

A Single Bout of Fatiguing Aerobic Exercise Induces Similar Pronounced Immunological Responses in Both Sexes

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Introduction: Physical exercise can acutely and chronically modulate immunological responses. Women and men have different innate and adaptive immune responses, and in this sense, these two groups may also have different acute immunological responses induced by exercise. In addition, it is essential to understand further whether the effects of physical exercise on the immune system responses depend on sex because limited scientific evidence on this topic is available. This information may allow athletes and coaches to improve the training process, mainly to understand if the physiological impact of given training stimuli in women is similar to that in men.

Objective: The present study aimed to investigate the acute effects of continuous submaximal exercise until fatigue on physiological and immunological parameters in amateur female and male runners.

Methods: This study included 18 female and 15 male volunteers. Each participant visited the laboratory on four consecutive days. The first visit consisted of medical history taking and explaining the study design. On the second visit, the participants were subjected to an incremental test to determine their maximal rate of oxygen consumption (VO_{2max}) that was required to prescribe the intensity of the submaximal exercise protocol. On the third visit, the fatiguing exercise protocol was performed at 77%–80% of the VO_{2max}. During this submaximal exercise, the heart rate, rating of perceived exertion (RPE), and blood lactate were recorded. Blood samples were collected before, immediately after, and 1 h after the fatiguing protocol to analyze the plasma levels of cytokines and creatine kinase (CK) and to count leukocytes. Finally, on the fourth visit, the participants underwent physical evaluations to measure their body composition using dual-energy X-ray absorptiometry (DXA) imaging.

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

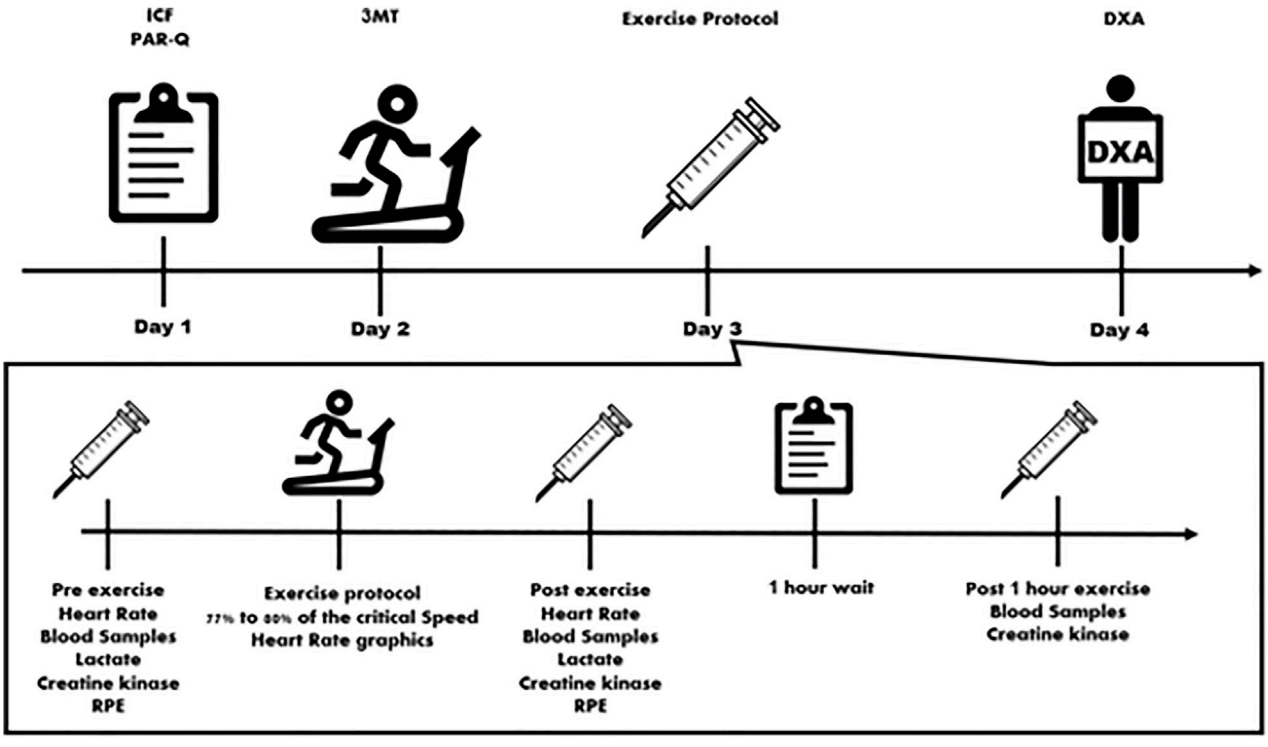
Received: *11 December 2021*
Accepted: *05 May 2022*
Published: *08 June 2022*

Citation:
Lobo LF, Morais MGd, Marcucci-Barbosa LS, Martins-Junior FdAD, Avelar LM, Vieira ELM, Aidar FJ, Wanner SP, Silva LS, Noman MC, Camargos BM, Freitas KM, Gonçalves WA, Pinho V and Nunes-Silva A (2022) A Single Bout of Fatiguing Aerobic Exercise Induces Similar Pronounced Immunological Responses in Both Sexes. Front. Physiol. 13:833580. doi: 10.3389/fphys.2022.833580

Results: The average ages of the female and male groups were 34.2 ± 3.7 and 30.5 ± 4.3 years old, respectively. The female group ran 57 ± 27 min, while the male group ran 52 ± 15 min before fatiguing. In the female group, when comparing before and after the submaximal exercise, marked increases were observed in the following variables: heart rate (from 68.5 to 180.4 bpm), RPE (from 3.6 to 8.2), lactate (from 2.1 to 4.49 mmol/L), and CK (from 89.5 to 126.3 U/L). In addition, the female group showed an increased number of total leukocytes (from 7222.3 to 11162.9 × 10^6/μl), neutrophils (from 4,403 to 6,480 × 10^6/μl), and lymphocytes (from 2,342 ± to 3,562 × 10^6/μl) from pre- to post-submaximal exercise. In the male group, similar elevations in psychophysiological variables were observed, as evidenced by comparing the heart rate (from 52.8 to 184.1 bpm), RPE (from 0.0 to 8.9), lactate (from 2.7 to 7.2 mmol/L), and CK (from 106.2 to 165 U/L) before and after the submaximal exercise. The male group also showed an augmented number of total leukocytes (from 6,245 to 8,050 × 10^6/μl), neutrophils (from 3,335 to 4,128 × 10^6/), and lymphocytes (from 2,191 to 3,212 × 10^6/μl) when comparing pre- and post-submaximal exercise. There were no differences in the changes between women and men for these parameters.

Conclusion: The aerobically fatiguing exercise protocol induced pronounced changes in the heart rate, plasma levels of lactate and CK, total leukocyte count, especially the number of neutrophils and lymphocytes, in both sexes. The fatiguing exercise protocol also changed the plasma levels of IL-6 and IL-10 in the female and male groups. Under the present conditions, the physiological changes induced by fatiguing submaximal exercise, including the immunological changes, were not influenced by sex. This study shows that the same aerobic physical exercise can alter immunological parameters in women and men, and this response is similar between sexes.

Keywords: immune response, myokines, skeletal muscle tissue, physical exercise (running), immunomodulation



GRAPHICAL ABSTRACT |

1 INTRODUCTION

Regular physical exercise improves the outcome of many of the emerging and increasingly prevalent clinical diseases. A massive body of recent findings demonstrates that one of the most important factors in this scenario is acute and chronic immunomodulation induced by exercise (Pedersen and Hoffman-Goetz, 2000). The immune system appears to be activated by physical stressors, and the immune/inflammatory response has become an essential component of physical training monitoring for athletes and recreationally active individuals. This low-grade inflammation status is associated with several types of obesity-related diseases such as diabetes, cardiovascular disease, cirrhosis, and cancer. Indeed, the literature suggests that control of this pathology-related inflammation can in part be ascribed by the release of immunogenic myokines (Bay and Pedersen, 2020).

Understanding the immune system is also crucial for promoting a healthy lifestyle, which includes adequate training, sufficient recovery, and a good nutrition. Plenty of evidence indicates that regular moderate-intensity physical exercise stimulates the immune system, culminating in protection against diseases, such as diabetes, hypertension, obesity, and many cancers (Pedersen and Febbraio, 2008; Pedersen and Febbraio, 2012). On the other hand, a sedentary lifestyle reduces life expectancy.

The modulation of the immune system by physical exercise, depends on important mediators, such as the cytokines, as well as the number of circulating leukocytes and their subpopulations, such as neutrophils, lymphocytes, monocytes, and eosinophils (Pedersen and Hoffman-Goetz, 2000; Brandt and Pedersen, 2010). These cytokines, which are produced and released by many cell types during and after physical exertion, mediate the beneficial effects of exercise on health. Cytokines can also influence metabolism and modify the production of cytokines in other tissues and organs, thus playing a fundamental role in regulating homeostasis and modulating the body's defense against chronic diseases (Gleeson et al., 2011; Benatti and Pedersen, 2015; Febbraio 2017). Many studies have shown increased levels of plasma concentrations of cytokines, such as interleukin-(IL)6, interleukin-(IL)10, tumor necrosis factor (TNF)α, and irisin after vigorous exercise (Peake et al., 2015; Blizzard LeBlanc et al., 2017). In addition, evidence from experimental studies in humans revealed elevated plasma concentration of several other cytokines, including IL-6, IL-8, IL-10, IL-15, CC-chemokine ligand (CCL)2, IL-1 receptor antagonist, calprotectin S100A9, and vascular endothelial growth factor (VEGF) (Hoffmann and Weigert, 2017). These changes have been observed in response to strength training (Fortunato et al., 2018; Marcucci-Barbosa et al., 2020) but also to endurance exercises that include both cycling (Gleeson, 2007; Zhao et al., 2012) and running (Kakanis et al., 2010; Marcucci-Barbosa et al., 2020). In general, systemic cytokine responses are more pronounced after exercises generating greater muscle damage, such as downhill running, eccentric exercise, and resistance training (Paulsen et al., 2012).

These findings have arisen from studies investigating men, likely because experimental studies in women are complex due to greater hormonal variations and specific responses caused by the menstrual cycle. As female physiology changes considerably during the month, some authors have claimed the need for studies on the particularities of physical exercise, sports performance, and immune system responses in women (Giraldo et al., 2008; Northoff et al., 2008; Giraldo et al., 2009). In this sense, the differences in immunological responses between men and women are probably influenced by biological factors (Katie and Flanagan, 2016). However, it is challenging to compare female and male data from the published studies, because few studies investigated the female population. Moreover, it is important to subject men and women to the same experimental design/exercise protocol to compare the immunological response between sexes.

Advanced knowledge of the biological sex differences in physiological responses will allow athletes and coaches to optimize the training process by understanding the physiological perturbations induced by training stimuli in women and men and how their body adapts/recovers after that. This knowledge will also help to understand possible sex-related differences in the ability of regular exercise to prevent disease occurrence. Given the limited scientific evidence comparing the exercise-induced immunological response between women and men, the present study investigated the acute effects of continuous submaximal exercise on physiological and immunological parameters in well-trained female and male runners. Herein, we hypothesized that the intense physical exercise could alter immunological markers in young adults and these alterations may show some differences between sexes.

2 METHODS

2.1 Study Design

Each participant visited the laboratory four times, being one daily visit across consecutive days. The first visit consisted of history taking and explaining the study objectives and design. In addition, the participants had the opportunity to request clarifications about the research before signing the informed consent form and completing the Physical Activity Readiness Questionnaire (PAR-Q). On the second visit, the participants performed an incremental exercise test to determine the maximum rate of oxygen consumption (VO_{2max}) that was required to prescribe the intensity of the fatiguing exercise. On the third visit day, the fatiguing exercise protocol at approximately 77%–80% of the VO_{2max} and the entire data collection were conducted. Finally, on the fourth visit, the participants underwent a physical evaluation to determine their body composition (i.e., percent body fat, body mass and height, and bone mineral density) using dual-energy X-ray absorptiometry (DXA) imaging.

2.2 Participants

Eighteen (18) women and fifteen (15) men took part in this study. The inclusion criterion for the female and male

participants in this study was the ability to run 10 km in <50 min and <45 min, respectively, as indicated by their performance in a race taking place in the 6 months preceding the day of data collection. In addition, the volunteers could not have reported musculoskeletal injuries in the lower limbs and pelvis in the 6 months before the experiments. The participants agreed to the following recommendations for participation: do not consume alcohol; do not perform strenuous exercise; do not take anti-inflammatory or analgesic drugs; and do not consume either anabolic steroids or nutritional supplements.

The data was collected by a physician instructed to interrupt the tests if, according to guidance from the American College of Sports Medicine (ACSM, 2014), one of the following aspects has been observed: angina or angina-like symptoms; increased chest pain; an inability of the heart rate to increase with exercise; any physical or verbal manifestations of extreme fatigue; loss of movement quality; a request to stop the exercise; or test equipment failure. During this study, no volunteer presented angina-like symptoms, chest pain, or inappropriate heart rate responses. This study was approved by the Human Research Ethics Committee of the Federal University of Ouro Preto (UFOP), protocol number 1.881.170 (CAAE: 60064216.5.0000.5150). The volunteers signed an informed consent form, which stated they could quit participating in this project at any time. All experimental procedures were done in accordance with the "Guidelines and Regulatory Norms for Research Involving Human Beings" of the National Health Council (Resolution 466/2012).

The sample size calculation was performed a priori using data from pilot experiments (n = 5) investigating the effect of a fatiguing exercise session on the number of circulating leukocytes. The effect size (i.e., partial eta-squared; η_p^2) for time points—before, immediately after, and 1 h after the exercise—corresponded to 0.253. We then used the GPower software (v 3.1.9.7) to calculate the required sample size according to the following parameters: ANOVA (repeated measures, within-between interaction) with two groups (males vs. females) and the three time points mentioned above, alpha error = 0.05, power = 0.95, correlation between repeated measurements = 0.68; effect size = 0.253, and non-sphericity correction = 1. This calculation indicated that a total of 28 participants were needed.

2.3 Incremental Running Tests

An incremental exercise test was performed on a treadmill (Centurion 300 Micromed) to determine the participants' cardiorespiratory capacity (VO_{2max}). The protocol started with 3 min of preparatory activity at a speed of 4 km/h and an incline of 1%, and then the speed was increased to 6 km/h while the incline was kept constant at 1%. During the test, the speed was increased by 0.1% every 30 s until voluntary fatigue (Castagna et al., 2009). Gas analysis was performed using an ergospirometer (Metalyzer 3B Cortex). VO_{2max}, respiratory exchange rate, heart rate, electrocardiogram, blood pressure, and perceived exertion were measured during this fatiguing exercise protocol. A medical cardiologist carried out the test at a sports medicine clinic accompanied by a researcher who conducted a brief interview.

2.3.1 Fatiguing Exercise Protocol

On the third visit, the participants arrived at the lab after having their habitual breakfast. After a 5-minute warm-up at <5 km/h, the speed at which the participants should exercise was adjusted on the treadmill dashboard. The treadmill automatically increased the speed until reaching the individual prescribed speed. The participants performed the constant exercise at an intensity corresponding to 77%–80% of VO_{2max} until they had voluntarily fatigued (e.g., inability to maintain the predetermined speed or a score of 10 on the perceived exertion scale) or asked to stop exercising (Miranda-Castro et al., 2022). Throughout the exercise protocol, the volunteers could drink water *ad libitum* but did not have access to information such as speed, running time, and heart rate.

2.4 Heart Rate and Rating of Perceived Exertion

The participant's heart rate was monitored throughout the fatiguing exercise protocol using a chest strap heart rate monitor (Polar m600 or V800). FlowSync version 3.0 (Polar®) was used to generate graphs of the heart rate values attained during the submaximal exercise. The maximum heart rate values were derived from the following equation [208 - (0.7 × age)]. The RPE was obtained on a 10-point Borg' scale (CR-10, Borg, 1998). During the 1 h that followed the submaximal exercise protocol, the volunteers did not perform any physical activity. RPE was collected only before and immediately after the voluntary fatigue. This scale measures the perceived effort related to the exercise and has been widely used as a marker of intensity (Borg, 1998).

2.5 Blood Sample Collection

A qualified health professional collected, transported, and stored the blood samples. Before exercise, immediately after, and again 1 h after the fatiguing exercise protocol, the participants' peripheral venous blood was collected through venipuncture in alternate arms using two vacutainer tubes (5 ml each) containing heparin as an anticoagulant. One of the tubes (fresh blood) was used for counting white blood cells and their subpopulations, while the other tube was centrifuged and stored for later analysis of CK and cytokines. The hematocrit and hemoglobin data from the blood count were used to correct the blood parameters by changes in the plasma volume, such as the plasma levels of creatine kinase and cytokines.

2.6 Lactate Concentration Analyses

Fingertip capillary blood samples were collected using a lancet to pierce the finger. The lactate concentrations in these samples were measured by reflectance photometry before and immediately

TABLE 1 | Characterization of the female and male groups. The absolute values and means of each characteristic are shown.

	Male				Female				
	Minimum	Mean	Maximum	S.D.	Minimum	Mean	Maximum	S.D.	*p* =
Age (years)	23.0	30.5	39.0	±4.3	25.0	34.2	40.0	±3.9	0.018*
Body mass (kg)	61.3	71.6	82.5	±6.7	49.1	57.8	72.2	±6.4	0.0001*
Muscle mass (%)	49.2	55.6	63.9	±4.1	61.4	69.6	77.0	±4.6	0.0001*
Body fat (%)	9.4	18.1	25.3	±4.5	19.5	27.3	35.7	±5.0	0.0001*
Lower limb muscle mass (kg)	17.2	19.4	22.8	±1.8	11.7	14.2	18.7	±1.9	0.0001*
Resting heart rate (bpm)	41.0	53.0	61.0	±5.0	56.0	68.5	93.0	±10.1	0.0001*
VO_2 (ml·kg·min)	48.9	54.2	71.3	±5.5	31.0	38.2	44.0	±3.6	0.0001*

*$p < 0.05$ *significantly different comparing female and male groups.*

after exercise, using reagent test strips inserted into a portable lactometer (*AccutrendPlus, Roche Diagnostic*). This measurement was performed within a maximum of 1 min after capillary blood collection.

2.7 Creatine Kinase Analysis

Plasma sample that was stored in a 1.5 ml *Eppendorf* in a −80°C freezer was used for CK analysis. The CK levels were measured at the Pilot Laboratory of Clinical Analysis (LAPAC) at the School of Pharmacy of the Federal University of Ouro Preto (UFOP). This analysis was conducted using a CK liquid reagent on an automated high-throughput chemistry analyzer (COBAS Integra 400-plus, Roche Diagnostics, United States).

2.8 Cytometric Bead Array Analysis for Measuring Cytokines

Plasma sample that was stored in a 1.5 ml *Eppendorf* in a −80°C freezer was used for cytokines analysis. The levels of Il-2, IL-4, IL-6, IL-10, TNF-α, and IFN-γ were measured using a *BD ™Cytometric Bead Array Kit* (CBA, BD Biosciences, San Diego, United States). The samples were diluted 1:5 in the test diluent. In parallel, a series of nine standard dilutions were prepared to obtain the standard curve. Mixed beads of cytokine catches were added, and the samples were incubated at 25°C in the dark for 90 min. The samples were then washed with a washing buffer and centrifuged for 7 min at 600 g at 18°C. The supernatant was discarded, and the beads were incubated with eight anti-cytokine antibodies conjugated with phycoerythrin at 25°C for another 90 min in the dark. The beads were then resuspended in wash buffer and immediately analyzed on a FACScan ™ flow cytometer. These measurements were performed in the Interdisciplinary Laboratory for Medical Research at the Faculty of Medicine of the Federal University of Minas Gerais.

2.9 Full Blood Count Analysis

The counting of the immune cells and their subpopulations was performed in a fresh blood sample on the same day of the submaximal exercise protocol. The analysis was carried out by a commercial laboratory hired to conduct this examination. The number of white cells and their subpopulations, as well as the hematocrit and hemoglobin concentration, were measured (Fortunato et al., 2018).

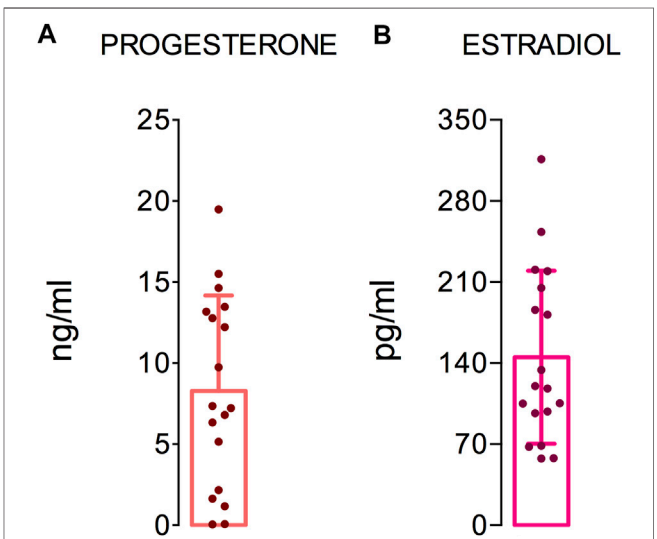


FIGURE 1 | Ovarian hormones levels. The graphs show the hormone levels in 18 women. The dashed (red) and dotted (black) lines show the reference values of progesterone and estradiol for the luteal and follicular phase of the menstrual cycle, respectively. The median, minimum, maximum, and first and third quartile values are shown.

2.10 Dual-Energy X-Ray Absorptiometry Imaging

The assessment of body composition using DXA imaging is increasingly common in the scientific community. This method allows accurate measurements of total body lean mass, lower limb region muscle mass, and the relative lean mass index, with high correlations reported with computed tomography and image resonance and lower radiation emission compared to computed tomography (El Maghraoui and Roux, 2008). In this study, DXA was performed by a qualified and experienced professional using a GE/Healthcare Model iDXA Densitometer (serial number ME + 210584) in a private hospital in the city of Belo Horizonte (Minas Gerais, Brazil). Body mass, height, and composition (bone mineral density, muscle mass, and percent body fat) were measured.

2.11 Statistical Analysis

GraphPad Prism version 6.0 was used to test the normality of the data. D'Agostino & Pearson tests were applied with

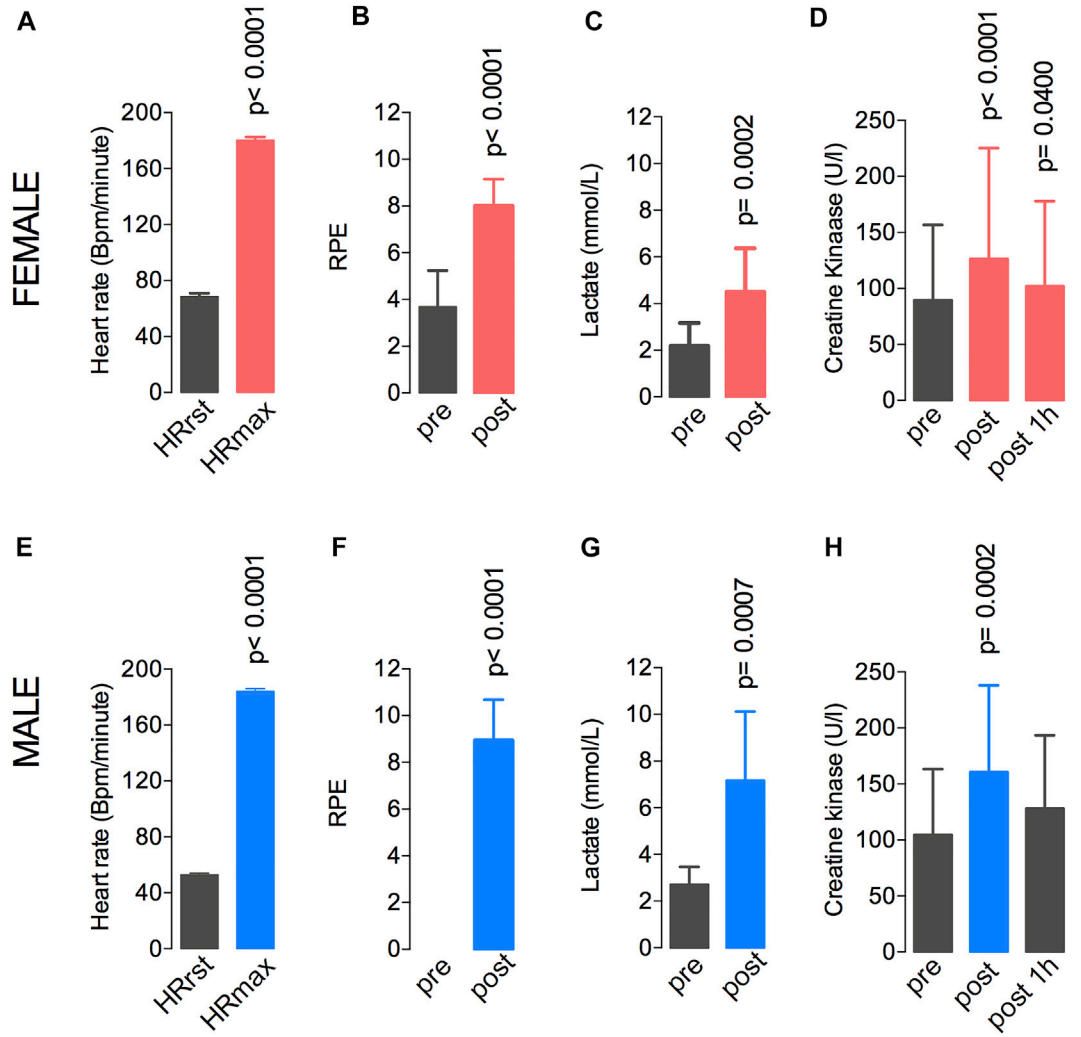


FIGURE 2 | A single session at 77%–80% of the VO_{2max} resulted in elevated heart rate **(A,E)**, RPE **(B,F)**, lactate levels **(C,G)**, and creatine kinase levels **(D,H)** in both male and female groups. Data are shown as means and standard deviations, with $p < 0.05$ indicating statistical significance. *Difference between time-points. RPE, rate of perceived exertion.

$\alpha = 0.01$. Wilcoxon tests were used for statistical analysis. Data with more than two time points that showed a normal distribution were analyzed by two-way mixed-design analysis of variance (ANOVA), with repeated measures used to compare intragroup data. The results were expressed as means ± standard deviations, with p values < 0.05 considered statistically significant.

3 RESULTS

3.1 General Participant Characteristics and Physiological Data of the Participants

Table 1 shows the average and standard deviation values of general characteristics and physiological data for women and men participants. In the male group, the average age was 30.5 ± 4.3 years, weight was 71.6 ± 5.9 kg, percent body fat was 18.1 ± 4.5%, total muscle mass was 55.6 ± 4.1 kg, whereas lower limb muscle mass was 19.4 ± 1.8 kg. These values in the female group were 34.2 ± 3.7 years, 57.8 ± 6.2 kg, 27.3 ± 4.8%, 40.0 ± 4.1 kg, and 14.1 ± 1.6 kg, respectively. The VO_{2max} was also recorded during the incremental exercise test and corresponded to 54.2 ± 5.5 and 38.2 ± 3.6 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$ in the male and female groups, respectively (**Table 1**).

3.2 Ovarian Hormone Levels

Women were tested in the same phase of the menstrual cycle to ensure that the fluctuations in hormone levels did not interfere with the immunological responses investigated. Therefore, we evaluated the ovarian hormones in the female group (**Figure 1**). The mean plasma levels of progesterone and estradiol were 8.2 ± 5.7 ng/ml (**Figure 1A**) and 145.1 ± 72.6 pg/ml (**Figure 1B**),

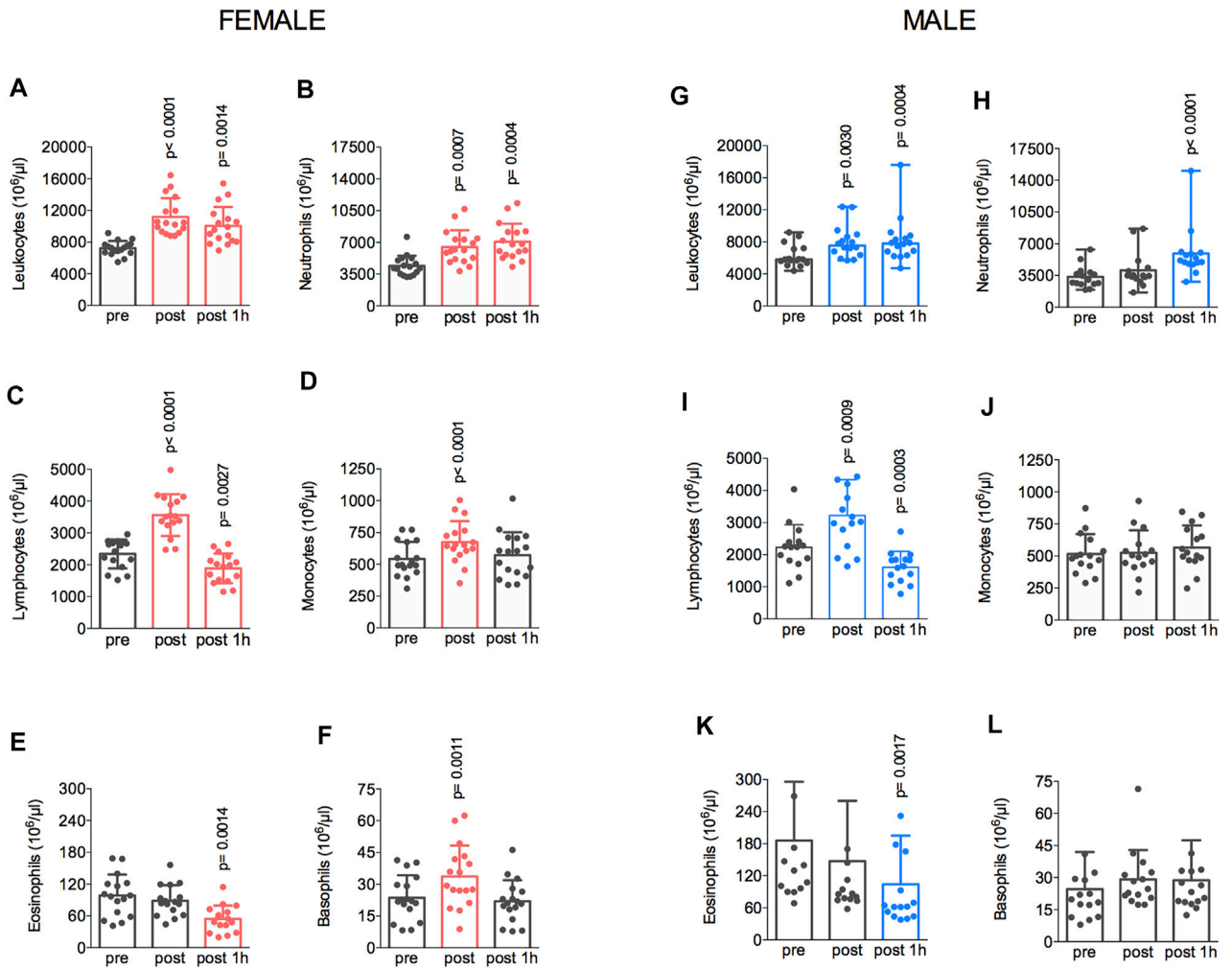


FIGURE 3 | The exercise protocol resulted in elevated total leukocyte **(A,G)** and lymphocytes **(C,I)** count immediately after the session. Neutrophil **(B,H)** count increased at 1 h post-test in both male and female groups. Monocytes **(D,J)** and basophils **(F,L)** count also increased 1-h post-test in the female group. Eosinophils **(E,K)** reduced at 1 h post-test in both male and female groups. Data are shown as means and standard deviations, with $p < 0.05$ indicating statistical significance. *Difference between time-points.

respectively. According to these data, the women were in the luteal phase of their menstrual cycle.

3.3 Fatiguing Aerobic Exercise-Induced Physiological Changes

The heart rate, the rate of perceived exertion (RPE), and the plasma levels of lactate and creatine kinase were evaluated during the fatiguing exercise on the treadmill in both groups (**Figure 2**). In the female group, between the pre-test and post-test, the heart rate increased from 68.5 to 180.4 bpm (**Figure 2A**), the RPE increased from 3.6 ± 1.5 to 8.2 ± 1.1 (**Figure 2B**), the lactate level increased from 2.1 ± 0.9 to 4.4 ± 1.8 mmol/L (**Figure 2C**), and the CK levels increased from 65.7 ± 67.1 to 96.9 ± 98.9 U/L (**Figure 2D**). In the male group, the heart rate increased from 52.8 to 184.1 bpm (**Figure 2E**). The RPE increased from 0.0 ± 0.0 to 8.9 ± 1.8 (**Figure 2F**), the lactate level increased from 2.7 ± 0.8 to 7.2 ± 2.9 mmol/L (**Figure 2G**), and the CK levels increased from 106.2 ± 60.6 to 165 ± 78.3 U/L (**Figure 2H**). Also, the expected exercise-induced changes in the heart rate variability (HRV) data were observed in the male participants. These changes included reduced parasympathetic-related indices (i.e., RMSSD and HF band; Shaffer and Ginsberg, 2017) and lower total power after the fatiguing exercise (**Supplementary Table S1**).

3.4 Effects of Fatiguing Aerobic Exercise on the Number of Leukocytes and Their Subpopulations

The fatiguing exercise protocol at 77%–80% of the VO_{2max} changed the count of circulating immune cells in both groups. The changes in cell counts from the pre-exercise to post-exercise, from the post-exercise to 1 h after, and from the pre-exercise to 1 h after are shown in **Figure 3**. In the female group, an increase

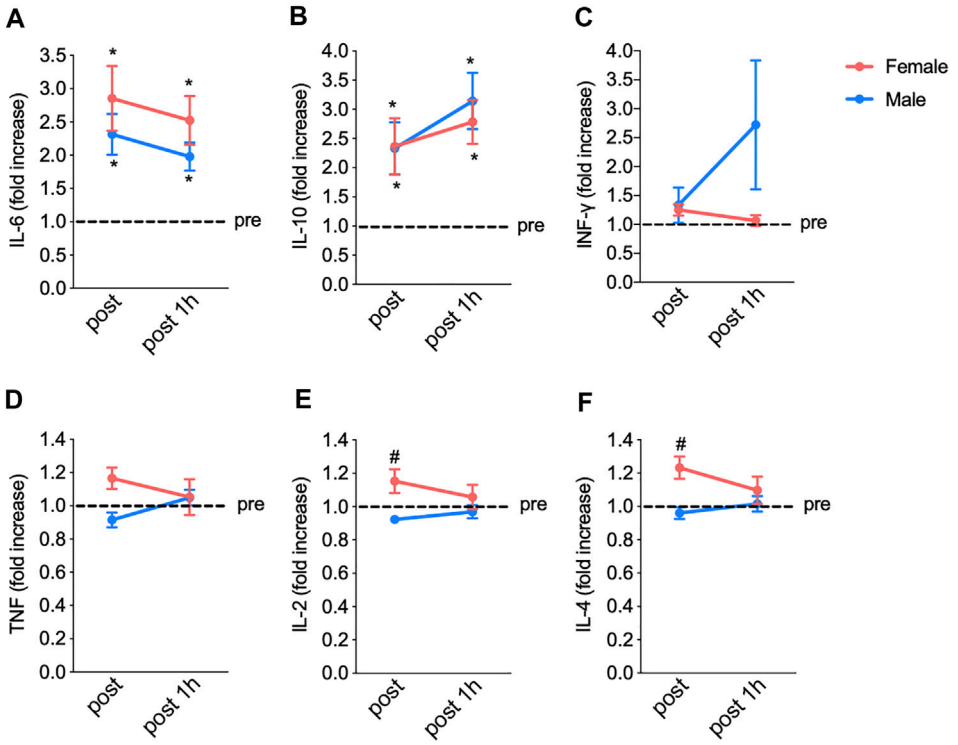


FIGURE 4 | The fatiguing exercise protocol results in increased interleukin IL-6 **(A)** and IL-10 **(B)** levels immediately after the exercise compared to pre-test values in both groups. No significant changes in INFγ **(C)**, TNFα **(D)**, IL-2 **(E)**, and IL-4 **(F)** concentrations were observed. Data are shown as means and standard deviations, with $p < 0.05$ indicating statistical significance. IFN, interferon; TNF, tumor necrosis factor; IL, interleukin.

in the total leukocyte count was also observed between pre- and post-exercise (7,222 ± 910 vs. 11,162 ± 2,374 × 10^6/μl) and the cell number remained elevated at 1 h after the exercise (10,040 ± 2,395 × 10^6/μl) (**Figure 3A**). Increased counts of neutrophils were also observed at post-exercise (4,403 ± 1,122 vs. 6,480 ± 1,860 × 10^6/μl) (**Figure 3B**). The number of lymphocytes also increased (2,342 ± 456 vs. 3,562 ± 655 × 10^6/μl) after the treadmill running and then decreased significantly (1,887 ± 465 × 10^6/μl) (**Figure 3C**) at 1 h after the exercise compared to the pre-exercise value. The monocytes count also increased when comparing pre- and post-exercise (542 ± 134 vs. 674 ± 165 × 10^6/μl) (**Figure 3D**). The eosinophil count did not change from pre- to post-exercise (98 ± 39 vs. 88 ± 29 × 10^6/μl), but it reduced (54 ± 25 × 10^6/μl) 1 h after the treadmill running (**Figure 3E**). Finally, the basophils count also increased from 23 ± 10 to 33 ± 14 × 10^6/μl at post-exercise relative to pre-exercise values (**Figure 3F**). In the male group, compared to the pre-exercise, the post-exercise total leukocyte count was increased from 6,245 ± 1,454 to 8,050 ± 2,170 × 10^6/μl, and it remained elevated at 1 h after the exercise (8,191 ± 3,106 × 10^6/μl) (**Figure 3G**). The neutrophil count also increased from 3,335 ± 1,245 to 4,128 ± 2,031 × 10^6/μl (**Figure 3H**), whereas the lymphocyte counts also increased from pre- to post-exercise (from 2,191 ± 716 to 3,212 ± 1,153 × 10^6/μl), and then it reduced significantly (1,579 ± 496 × 10^6/μl) at 1 h after the test, compared to the pre-exercise value (**Figure 3I**). No changes were observed for the number of monocytes (504 ± 154 vs. 652 ± 165 × 10^6/μl) (**Figure 3J**). The eosinophil count decreased from 189 ± 113 to 107 ± 93 × 10^6/μl 1 h after relative to pre-exercise (**Figure 3K**). Finally, no changes were observed for basophil counts (22 ± 10 vs. 29 ± 14 × 10^6/μl) (**Figure 3L**).

3.5 Effects of Fatiguing Aerobic Exercise on Plasma Cytokine Levels

These results reinforced that running on a treadmill indeed modulates immunological responses, as further evidenced by the changes in the plasma levels of cytokines in both women and men (**Figure 4**). In the female group, the fatiguing exercise protocol increased the levels of IL-6 (from 1.0 to 2.8-fold, from pre-exercise to post-exercise) (**Figure 4A**) and IL-10 (from 1.0 to 2.3-fold) (**Figure 4B**); these levels remained elevated (2.5- and 2.8-fold, respectively) at 1 h after the exercise. In addition, no exercise-induced changes were observed in the plasma levels of IFN-γ, (**Figure 4C**), TNF-α (**Figure 4D**), IL-2 (**Figure 4E**), and IL-4 (**Figure 4F**). In the male group, the fatiguing exercise increased the levels of IL-6 (from 1.0 to 2.3-fold) (**Figure 4A**) and IL-10 (from 1.0 to 2.3); these levels remained elevated (2.0- and 2.1-fold, respectively) at 1 h after the exercise. No exercise-induced changes were observed in the levels of IFN-γ (**Figure 4C**), TNFα (**Figure 4D**), IL-2 (**Figure 4E**), and IL-4 (**Figure 4F**). A two-way ANOVA analysis also showed the differences between female and male groups in the IL-2 and IL-4 levels when comparing pre-test and post-test.

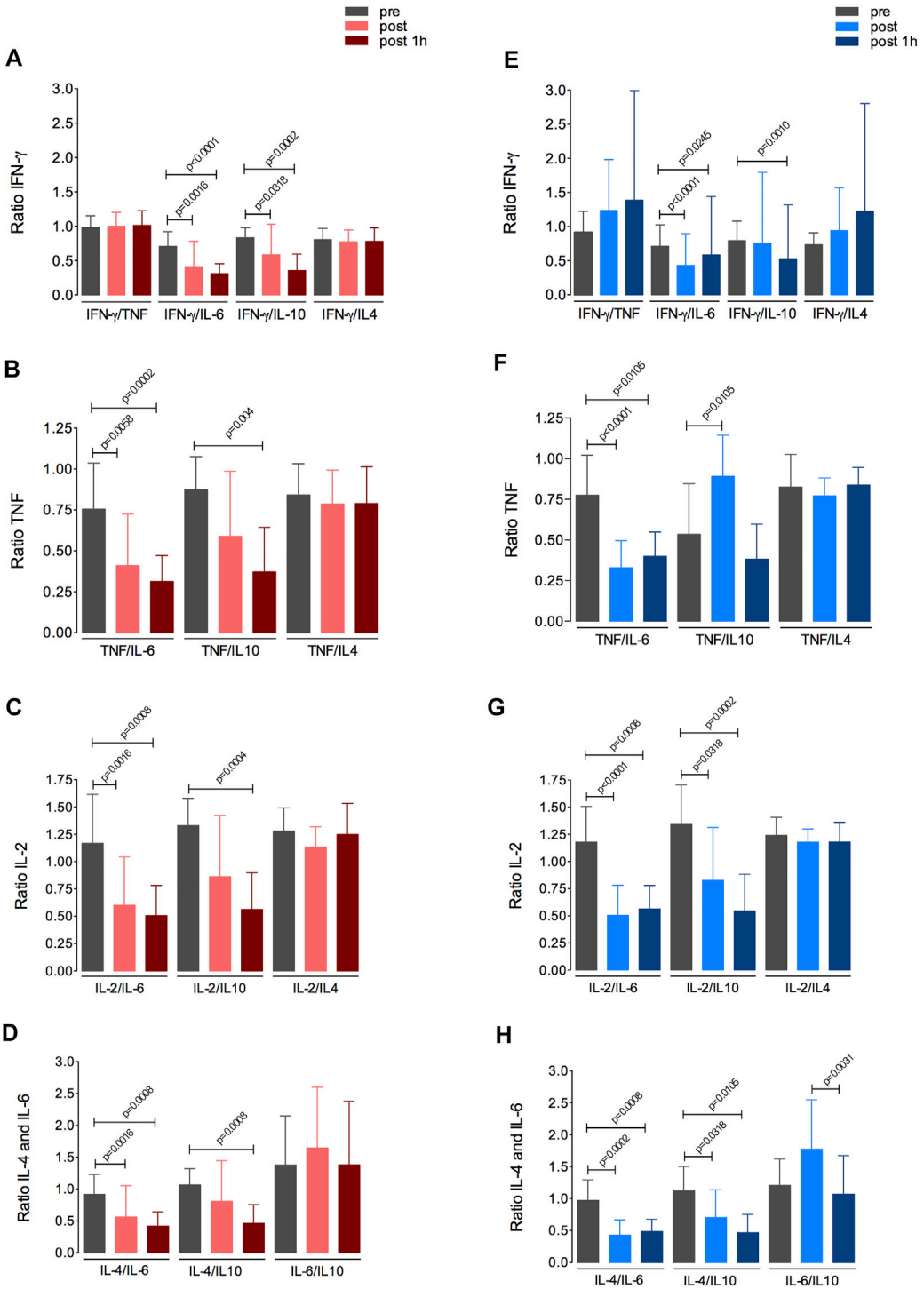


FIGURE 5 | Changes in IFNγ, TNFα, IL-6, IL-2, IL-4, and IL-10 levels in response to the fatiguing exercise protocol. Changes in the levels of IFNγ **(A,B)**, TNFα **(C,D)**, IL-2 **(E,F)**, IL-4 **(G,H)** in the female and male groups are shown. IFN, interferon; TNF, tumor necrosis factor; IL, interleukin.

3.6 Effects of Fatiguing Aerobic Exercise on IFN-γ, TNFα, IL-6, IL-2, IL-4, and IL-10 Levels

The possible relationships between the changes in IFN-γ, TNFα, IL-6, IL-2, IL-4, and IL-10 levels in response to the fatiguing exercise were investigated in both sex groups (**Figure 5**). The changes in the levels of these cytokines following the fatiguing aerobic exercise were similar between women and men. In addition, in both groups, IL-6 appeared to modulate the cytokine response, as IL-6 levels were directly related to those of IFN-γ (**Figures 5A,B**), TNFα (**Figures 5C,D**), IL-2 (**Figures 5E,F**), and IL-4 (**Figures 5G,H**).

4 DISCUSSION

Most publications investigating the relationship between physical exercise and immune response involve male participants, likely because experimental studies in women are more complex due to large hormonal fluctuations and specific responses during the menstrual cycle. In this sense, few studies have evaluated the effect of a single bout of aerobically fatiguing exercises on the immunological response in both sexes. The main results of the present study were: 1) the aerobic exercise induced pronounced changes in leukocyte counts in both sexes; 2) the aerobic exercise also induced important changes in plasma cytokine levels in both sexes; 3) the changes and their magnitudes were similar between the sexes; and finally, 4) IL-6 cytokine appears to play a regulatory role in the exercise-induced immune response in women and men.

The present study focused on comparing the immunological response of women and men after fatiguing treadmill running, and this is an emerging topic in the field of exercise immunology. The current findings may help understand possible immunological differences between female and male athletes and physically active individuals after a physical exercise session. Of note, the immune response is involved in several training-induced adaptations, including those occurring in skeletal muscles (Chazaud, 2020). Moreover, our findings are also crucial to understand whether biological sex modulates the role played by exercise in preventing or treating diseases associated with augmented systemic inflammation, such as obesity and type 2 diabetes (Pedersen, 2012).

As expected, a single session of aerobic exercise until fatigue produced marked changes in psychophysiological parameters. From pre- to post-exercise, we observed increased heart rate, RPE, plasma lactate levels, and circulating CK levels. All these responses agree with the findings of previous studies in which the individuals were subjected to a single session of vigorous exercise (Fortunato et al., 2018; Marcucci-Barbosa et al., 2020). In fact, the RPE and heart rate values at fatigue above 8 and 180 bpm, respectively, confirm that the women and men participants were exercising at their maximum or near-maximum effort when they stopped running. Interestingly, CK levels were already augmented immediately after the exercise, even though evidence suggests that this muscle damage peaks around 24 h after exercise on a treadmill (Hackney et al., 2019; Aloulou et al., 2020) or a soccer match-play (Silva et al., 2018). However, data are scarce on the differences in exercise-induced acute immune response between female and male groups.

Vigorous physical exercise modulates some fundamental aspects of the immune system, such as the number of circulating white blood cells and plasma levels of cytokines (Kakanis et al., 2010). However, data on sex differences in exercise-induced acute immune responses are scarce. Because women and men have different immunological responses to foreign and self-antigens and show distinctive innate and adaptive immune responses (Klein and Flanagan, 2016), we expected that they also should respond differently to a physical exercise session.

The present results showed that the fatiguing aerobic exercise induced pronounced changes in the number of white blood cells (**Figure 3**), but these changes were similar between sexes. Our findings agree with the observation that 90-min moderate-intensity cycling induced similar increases in immune cell counts between men and women not using contraceptives, except for a 38% greater lymphocyte response in these women. However, neutrophil, monocyte, and lymphocyte responses to exercise during the luteal phase in women using contraceptives were greater than those in men (Timmons et al., 2005). Moreover, our data also disagree with findings obtained in children and adolescents (Timmons et al., 2006). Exercise consisting of cycling for 60 min at 70%VO_{2max} increased the number of lymphocytes and CD3j, $CD16^+$, and $CD56^+$, with greater increases reported in adolescent girls than boys, but no differences between younger girls and boys. In addition, the exercise-induced increases in the counts of total leukocyte, lymphocyte, CD3j, $CD16^+$, and $CD56^+$ were at least 35% greater in girls than in boys with similar pubertal status (Timmons et al., 2006). Probably the difference between the results in these studies could be associated with the intensity of the exercise, once our volunteers exercised until fatigue.

Interestingly, our findings are in accordance with those provided by Gleeson et al. (2011). In the latter study, eighty physically active individuals (46 men, 34 women) trained on average 10 h/week at moderate-to-vigorous intensities; after that, differential leukocyte counts and lymphocyte subsets were determined. While the total blood leukocyte, neutrophil, monocyte, and lymphocyte counts did not differ between sexes, men had more B and NK cells. Consistent with the present results, the authors concluded that most aspects of immunity were similar between the sexes in an athletic population, with some differences in a few immune variables (Gleeson et al., 2011).

Finally, Abbasi et al. (2016) showed that running a half-marathon significantly increased total leukocyte count for 3 h post-exercise in both male and female athletes, with no sex-specific differences in the number of any immune cell population or total leukocytes. Neutrophil numbers and percentages were both significantly increased at 30 min and 3 h post-exercise, while the percentages of monocytes and lymphocytes were decreased at the same time points. The lymphocyte percentages returned to the pre-exercise levels at 24 h post-exercise. Once again, these

(sem virgula entre once e again) results did not show a difference between sexes.

Our findings are in line with the similar exercise-induced increase in plasma IL-6 between men and women not using contraceptives and with IL-6 comparisons made between sexes in a pediatric population (Timmons et al., 2005). Our analyses indicate that most cytokines responded similarly in women and men, except for IL-2 and IL-4. IL-2 is a cytokine signaling molecule that regulates the activities of leukocytes, often lymphocytes. IL-2 is part of the body's natural response to microbial infection, and in discriminating between foreign ("non-self") and "self." The major sources of IL-2 are activated $CD4^+$ T cells and activated $CD8^+$ T cells. IL-4 is a cytokine that functions as a potent regulator of immunity secreted primarily by mast cells, Th2 cells, eosinophils, and basophils. IL-4 is an important player in leukocyte survival under both physiological and pathological conditions, such as Th2 cell-mediated immunity, IgE class switching in B cells, and tissue repair and homeostasis through "alternative" macrophage activation. IL-4 is produced primarily by mast cells, T_h2 cells, eosinophils, and basophils (Liu et al., 2021).

It is important to mention here that the main aim of this study was to compare the immune response between female and male groups. The current statistical analyzes clearly show that the immune response between the female and male groups is similar. Only the IL-4 and IL-2 responded differently between groups. We reinforce that we tried to ensure that the female group was in the same phase of the menstrual cycle to avoid possible effects on the immune response. The novelty of this study is to clearly show that the same aerobic physical exercise protocol can alter immunological parameters in women and men and this response is similar between sexes.

The strongest aspect of this study is that the same physical exercise was used in male and female volunteers. Another important aspect is that the female group was in the same phase of the menstrual cycle. On the other hand, one important limitation of the currently study is the fact that we analyzed only the luteal phase of the menstrual cycle. From a practical perspective, these results suggest that the training process of immune system could be similar in both groups. As future perspectives, it is important to better understand the chronic application of training process on immune system and compare the responses between female and male population.

5 CONCLUSION

The aerobically fatiguing exercise protocol induced pronounced changes in the immune parameters—such as the total leukocyte count, especially the neutrophil and lymphocyte numbers—and in the plasmatic cytokine levels of both female and male groups. The results also show that these responses were similar between both sexes.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Universidade Federal de Ouro Preto (UFOP). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

The study was designed and written by MM, LL, and AN-S; data acquisiton, analysis, and interpretation during the manuscript preparations were carried out by LM-B, FJ, LA, EV, FJA, SW, LS, MN, BC, KF, WG, VP, MM, LL, and AN-S. All authors approved the final version of the article.

ACKNOWLEDGMENTS

We would like to thank Dr. Samuel Warnner from University of Minas Gerais, MD Marconi from the SPORTIF and EV from CAMH (Toronto).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.833580/full#supplementary-material

REFERENCES

Abbasi, A., de Paula Vieira, R., Bischof, F., Walter, M., Movassaghi, M., Berchtold, N. C., et al. (2016). Sex-specific Variation in Signaling Pathways and Gene Expression Patterns in Human Leukocytes in Response to Endotoxin and Exercise. *J. Neuroinflammation* 13, 289. doi:10.1186/s12974-016-0758-5

Aloulou, A., Duforez, F., Bieuzen, F., and Nedelec, M. (2020). The Effect of Night-Time Exercise on Sleep Architecture Among Well-Trained Male Endurance Runners. *J. Sleep. Res.* 29 (6), e12964. doi:10.1111/jsr.12964

Bay, M. L., and Pedersen, B. K. (2020). Muscle-Organ Crosstalk: Focus on Immunometabolism. *Front. Physiol.* 11, 567881. doi:10.3389/fphys.2020.567881

Benatti, F. B., and Pedersen, B. K. (2015). Exercise as an Anti-inflammatory Therapy for Rheumatic Diseases-Myokine Regulation. *Nat. Rev. Rheumatol.* 11, 86–97. doi:10.1038/nrrheum.2014.193

Blizzard LeBlanc, D. R., Rioux, B. V., Pelech, C., Moffatt, T. L., Kimber, D. E., Duhamel, T. A., et al. (2017). Exercise-induced Irisin Release as a Determinant of the Metabolic Response to Exercise Training in Obese Youth: the EXIT Trial. *Physiol. Rep.* 5. doi:10.14814/phy2.13539

Borg, G. (1998). *Borg's Perceived Exertion and Pain Scales*. Champaign, IL: Human Kinetics, 104.

Brandt, C., and Pedersen, B. K. (2010). The Role of Exercise-Induced Myokines in Muscle Homeostasis and the Defense against Chronic Diseases. *J. Biomed. Biotechnol.* 2010, 520258. doi:10.1155/2010/520258

Castagna, C., Impellizzeri, F., Cecchini, E., Rampinini, E., and Alvarez, J. C. B. (2009). Effects of Intermittent-Endurance Fitness on Match Performance in Young Male Soccer Players. *J. Strength Cond. Res.* 23, 1954–1959. doi:10.1519/jsc.0b013e3181b7f743

Chazaud, B. (2020). Inflammation and Skeletal Muscle Regeneration: Leave it to the Macrophages!. *Trends Immunol.* 41 (6), 481–492. doi:10.1016/j.it.2020.04.006

El Maghraoui, A., and Roux, C. (2008). DXA Scanning in Clinical Practice. *QJM An Int. J. Med.* 101 (8), 605–617. doi:10.1093/qjmed/hcn022

Febbraio, M. A. (2017). Health Benefits of Exercise - More Than Meets the Eye!. *Nat. Rev. Endocrinol.* 13, 72–74. doi:10.1038/nrendo.2016.218

Fortunato, A. K., Pontes, W. M., De Souza, D. M. S., Prazeres, J. S. F., Marcucci-Barbosa, L. S., Santos, J. M. M., et al. (2018). Strength Training Session Induces Important Changes on Physiological, Immunological, and Inflammatory Biomarkers. *J. Immunol. Res.* 2018, 1–12. doi:10.1155/2018/9675216

Giraldo, E., Garcia, J. J., Hinchado, M. D., and Ortega, E. (2009). Exercise Intensity-dependent Changes in the Inflammatory Response in Sedentary Women: Role of Neuroendocrine Parameters in the Neutrophil Phagocytic Process and the Pro-/anti-inflammatory Cytokine Balance. *Neuroimmunomodulation* 16, 237–244. doi:10.1159/000212384

Giraldo, E., Hinchado, M. D., Garcia, J. J., and Ortega, E. (2008). Influence of Gender and Oral Contraceptives Intake on Innate and Inflammatory Response. Role of Neuroendocrine Factors. *Mol. Cell. Biochem.* 313, 147–153. doi:10.1007/s11010-008-9752-2

Gleeson, M., Bishop, N. C., Stensel, D. J., Lindley, M. R., Mastana, S. S., and Nimmo, M. A. (2011). The Anti-inflammatory Effects of Exercise: Mechanisms and Implications for the Prevention and Treatment of Disease. *Nat. Rev. Immunol.* 11, 607–615. doi:10.1038/nri3041

Gleeson, M. (20071985). Immune Function in Sport and Exercise. *J. Appl. Physiology* 103, 693–699. doi:10.1152/japplphysiol.00008.2007

Hackney, A. C., Kallman, A. L., and Ağgön, E. (2019). Female Sex Hormones and the Recovery from Exercise: Menstrual Cycle Phase Affects Responses. *Biomed. Hum. Kinet.* 11, 87–89. doi:10.2478/bhk-2019-0011

Hoffmann, C., and Weigert, C. (2017). Skeletal Muscle as an Endocrine Organ: the Role of Myokines in Exercise Adaptations. *Cold Spring Harb. Perspect. Med.* 7, a029793. doi:10.1101/cshperspect.a029793

Kakanis, M. W., Peake, J., Brenu, E. W., Simmonds, M., Gray, B., Hooper, S. L., et al. (2010). The Open Window of Susceptibility to Infection after Acute Exercise in Healthy Young Male Elite Athletes. *Exerc. Immunol. Rev.* 16, 119–137. doi:10.1016/j.jsams.2010.10.642

Klein, S. L., and Flanagan, K. L. (2016). Sex Differences in Immune Responses. *Nat. Rev. Immunol.* 16, 626–638. doi:10.1038/nri.2016.90

Liu, C., Chu, D., Kalantar-Zadeh, K., George, J., Young, H. A., and Liu, G. (2021). Cytokines: From Clinical Significance to Quantification. *Adv. Sci.* 8, 2004433.doi:10.1002/advs.202004433

Marcucci-Barbosa, L. S., Martins-Junior, F. d. A. D., Lobo, L. F., de Morais, M. G., Aidar, F. J., Vieira, E. L. M., et al. (2020). The Effects of Strength Training Session with Different Types of Muscle Action on White Blood Cells Counting and Th1/Th2 Response. *Sport Sci. Health* 16, 239–248. doi:10.1007/s11332-019-00597-3

Miranda-Castro, S., Aidar, F. J., de Moura, S. S., Lobo, L. F., de Assis Dias Martins-Júnior, F., da Silva Filha, R., et al. (2022). The Curcumin Supplementation with Piperine Can Influence the Acute Elevation of Exercise-Induced Cytokines: Double-Blind Crossover Study. *Biology* 11, 573. doi:10.3390/biology11040573

Northoff, H., Symons, S., Zieker, D., Schaible, E. V., Schäfer, K., Thoma, S., et al. (2008). Gender- and Menstrual Phase Dependent Regulation of Inflammatory Gene Expression in Response to Aerobic Exercise. *Exerc. Immunol. Rev.* 14, 86–103.

Paulsen, G., Mikkelsen, U. R., Raastad, T., and Peake, J. M. (2012). Leucocytes, Cytokines and Satellite Cells: what Role Do They Play in Muscle Damage and Regeneration Following Eccentric Exercise? *Exerc. Immunol. Rev.* 18, 42–97. https://pubmed.ncbi.nlm.nih.gov/22876722/.

Peake, J. M., Della Gatta, P., Suzuki, K., and Nieman, D. C. (2015). Cytokine Expression and Secretion by Skeletal Muscle Cells: Regulatory Mechanisms and Exercise Effects. *Exerc. Immunol. Rev.* 21, 8–25. https://pubmed.ncbi.nlm.nih.gov/25826432/.

Pedersen, B. K., and Febbraio, M. A. (2008). Muscle as an Endocrine Organ: Focus on Muscle-Derived Interleukin-6. *Physiol. Rev.* 88 (4), 1379–1406. doi:10.1152/physrev.90100.2007

Pedersen, B. K., and Febbraio, M. A. (2012). Muscles, Exercise and Obesity: Skeletal Muscle as a Secretory Organ. *Nat. Rev. Endocrinol.* 8 (8), 457–465. doi:10.1038/nrendo.2012.49

Pedersen, B. K., and Hoffman-Goetz, L. (2000). Exercise and the Immune System: Regulation, Integration, and Adaptation. *Physiol. Rev.* 80, 1055–1081. doi:10.1152/physrev.2000.80.3.1055

L S. Pescatello, R. Arena, D. Riebe, and P. D. Thompson (Editors) (2014). *ACSM's Guidelines for Exercise Testing and Prescription*. 9th Ed. (Philadelphia: Lippincott Williams & Wilkins).

Shaffer, F., and Ginsberg, J. P. (2017). An Overview of Heart Rate Variability Metrics and Norms. *Front. Public Health* 5, 258. doi:10.3389/fpubh.2017.00258

Silva, J. R., Rumpf, M. C., Hertzog, M., Castagna, C., Farooq, A., Girard, O., et al. (2018). Acute and Residual Soccer Match-Related Fatigue: A Systematic Review and Meta-Analysis. *Sports Med.* 48 (3), 539–583. doi:10.1007/s40279-017-0798-8

Timmons, B. W., Hamadeh, M. J., Devries, M. C., and Tarnopolsky, M. A. (2005). Influence of Gender, Menstrual Phase, and Oral Contraceptive Use on Immunological Changes in Response to Prolonged Cycling. *J. Appl. Physiology* 99, 979–985. doi:10.1152/japplphysiol.00171.2005

Timmons, B. W., Tarnopolsky, M. A., Snider, D. P., and Bar-Or, O. (2006). Immunological Changes in Response to Exercise. *Med. Sci. Sports Exerc* 38 (2), 293–304. doi:10.1249/01.mss.0000183479.90501.a0

Zhao, G., Zhou, S., Davie, A., and Su, Q. (2012). Effects of Moderate and High Intensity Exercise on T1/T2 Balance. *Exerc Immunol. Rev.* 18, 98–114.

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BRIEF RESEARCH REPORT
published: 06 July 2022
doi: 10.3389/fphys.2022.855133

Potential Anticarcinogenic Effects From Plasma of Older Adults After Exercise Training: An Exploratory Study

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Edited by:
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Specialty section:
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

Received: 14 January 2022
Accepted: 23 May 2022
Published: 06 July 2022

Citation:
Peres A, Branchini G, Marmett B, Nunes FB, Romão PRT, Olean-Oliveira T, Minuzzi L, Cavalcante M, Elsner V, Lira FS and Dorneles GP (2022) Potential Anticarcinogenic Effects From Plasma of Older Adults After Exercise Training: An Exploratory Study. Front. Physiol. 13:855133. doi: 10.3389/fphys.2022.855133

Aim: To evaluate the impact of exercise training plasma on *in vitro* prostate cancer cell viability and proliferation.

Methods: PC3 prostate cancer cells were incubated with plasma obtained from young men with high and low physical fitness (PF) (high PF, $n = 5$; low PF, $n = 5$) and with the plasma collected from institutionalized older adults ($n = 8$) before and after multimodal exercise training. Cell viability and proliferation, mitochondria membrane polarization, reactive oxygen species (ROS) generation, and apoptosis were evaluated after the cell treatment with plasma. Systemic cytokines were evaluated in the plasma of institutionalized older adults submitted to an exercise training protocol.

Results: Plasma from high-PF men lowers both cell viability and proliferation after the incubation time. PC3 cells also presented lower cell viability and diminished rates of cell proliferation after the incubation with post-training plasma samples of the older adults. The incubation of PC3 cells with post-training plasma of older adults depolarized the mitochondrial membrane potential and increased mitochondrial reactive oxygen species production. Post-training plasma did not change apoptosis or necrosis rates in the PC3 cell line. Multimodal exercise training increased the plasma levels of IL-2, IL-10, IFN-α, and FGF-1 and decreased TNF-α concentrations in institutionalized older adults.

Conclusion: Adaptations in blood factors of institutionalized older adults may alter cell viability and proliferation by targeting mitochondrial ROS in a prostate cancer cell line.

Keywords: prostate cancer, immune response, aging, exercise training, inflammation, mitochondria

INTRODUCTION

Biological aging impacts several aspects of the host immune system, characterized by a chronic inflammatory state associated with an accumulation of senescent exhausted myeloid and lymphoid cells (Bauer and De la Fuente, 2013). The chronic increases in inflammatory molecules are associated with the etiology and clinical course of most age-related diseases and mortality, including several cancer types (Barbé-Tuana et al., 2020). In this sense, prostate cancer becomes more prevalent in aging men, once the enhancement in the innate and adaptive inflammatory response contributes to prostate carcinogenesis (Taverna et al., 2014).

Emerging data indicate that physical exercise may have positive effects on the prevention and treatment of prostate cancer (Kim et al., 2021). Both preclinical and clinical studies described several immunological and physiological exercise-mediated adaptations that may prevent or attenuate prostate cancer progression, including enhanced T-cell and natural killer functional activity as well as the modification of systemic biochemical molecules (Kim et al., 2021). Furthermore, changes in systemic inflammation and the composition of blood factors induced by exercise training adaptations may directly impact cancer cell viability. Past data highlighted the role of acute exercise-conditioned serum or plasma from young and aged humans in the decrease of cancer cell viability, indicating that changes in blood molecules (i.e., hormones, cytokines, and reactive oxygen species) contribute to the anticarcinogenic potential during acute bouts (Hwang et al., 2020; Soares et al., 2021; Orange et al., 2022).

Decreases in prostate cancer cell viability induced by pharmacological drugs are mediated by changes in mitochondrial functions (Seo et al., 2019). In fact, mitochondria are emerging players in the tumorigenic process by maintaining the energetic capabilities of cancer cells (Boland, Chourasia, and Macleod, 2013). In prostate cancer, it is now clear that mitochondria are involved in the malignant process, cell proliferation aggression, and metastasis formation (Zichri et al., 2021). Mitochondria dysfunction and reactive oxygen species (ROS) generation are important events to reduce cancer cell viability (Wu et al., 2011). Modifications in mitochondrial activity lead to the expression of the cell cycle inhibitor p53, cell cycle arrest at the G2/M phase, DNA fragmentation, and subsequent cell death induction (Wu et al., 2011). Furthermore, *in vivo* observations demonstrated that ROS could trigger tumor apoptosis through increasing lipid, protein, and DNA damage within the tumor (Xie et al., 1995). It is interesting to note that chemotherapeutic agents induce ROS-induced lipid peroxidation and apoptosis in tumor cells (Jana et al., 2014). Exercise changes the redox state in several tissues and may be an important non-pharmacological agent to contribute to the tumor cell's growth, acting as a preventive agent or as a rehabilitation tool. However, few studies evaluated the potential effects of exercise training to induce anticarcinogenic effects in the plasma of older adults (Soares et al., 2021). Furthermore, to date, no study focused on mitochondrial activity of cancer cells after exercised plasma treatment. This *in vitro* study evaluated the role of the older adult plasma submitted to multimodal exercise training in the viability and proliferation of immortalized prostate cancer androgen unresponsive PC3 cells.

METHODS

Participants

Eight institutionalized older adults (aged 73.38 ± 11.28 y; body mass index 27.8 ± 4.9 kg/m^2) living in a long-term facility in Porto Alegre City, south of Brazil, were considered. The inclusion and exclusion criteria were previously reported (Fraga et al., 2021). The sample size of older adults was limited to the individuals who lived in a single long-term facility and were able to perform exercise. Participants were not engaged in structured exercise training protocols for a period prior to 6 months before the trial. The Ethics Research Committee of Centro Universitario Metodista-IPA, Brazil, approved the current study number 3.376.078. All participants signed written informed consent before enrollment, and all procedures were in conformity with the Declaration of Helsinki. This study was not prospectively registered. Additionally, we collected venous blood samples from young lean men with high physical fitness (PF) (high PF: n = 5; age, 27.8 ± 5.6 y; body mass index, 24.3 ± 1.3 kg/m^2; VO_{2Peak} 49.7 ± 2.67 ml.kg.min) and low PF (low PF: n = 5; age, 29.8 ± 2.7 y; body mass index, 23.1 ± 2.1 kg/m^2; VO_{2Peak} 37.8 ± 3.1 ml.kg.min) as controls of the study. The criteria for being physically active were the completion of at least 3 h of endurance training per week, for a minimum of 3 years, and a peak oxygen consumption (VO_{2Peak}) of at least 45 ml/kg/min. Individuals who reported less than 100 min of physical activity per week and not engaging in regular exercise training for at least 2 years were defined as physically inactive/sedentary. All young participants were recruited from the general community of Porto Alegre/Brazil and performed a cardiopulmonary exercise (CPET) to determine their physical fitness. Detailed information regarding recruitment criteria was previously published in Dorneles et al. (2019). Physical fitness was evaluated by a CPET test as previously described Dorneles et al., 2019. In brief, the CPET was performed on an electric treadmill (Centurion 300; Micromed, Brasilia, Brazil) using a ramp protocol. Both the speed and incline of the treadmill gradually increased up to the maximum limit of the participant. Ventilatory and metabolic parameters were collected by respiration using a Metalyzer 3B (Cortex, Leipzig, Germany) and were analyzed after the mean of the data in eight respiratory cycles. The CPET system was calibrated before each test with respect to both airflow and O_2 and CO_2 analyzers. The average of the last 30 s of the test was used to determine the VO_2 peak.

Study Design and Training Protocol

Older adults participated in a multimodal exercise training (8 weeks, 2x/week, 60 min each session) supervised by a trained physiotherapist. The multimodal protocol used in the current study was based on a previous study (Pereira et al., 2018). The components of intervention are in adherence to the Consensus on Exercises Reporting Template (CERT) (**Supplementary Material S1**). The exercise intervention was intended to stimulate physiological, perceptual, and cognitive mechanisms. Therefore, the exercise sessions were planned to guarantee stimuli relevant to promoting simultaneous motor and cognitive stimulation and to incorporate challenging activities that induced the participants to mobilize several types of abilities.

Specifically, each session was divided into the four following moments: 1) warm-up (5 min), with stretching and active upper/lower limb exercises; 2) exercises focused on cardiovascular capacity, strength, balance/agility, and flexibility (25 min), which included walking, stationary gait, resistance exercises for the main upper/lower muscle groups, unipodal support with open/closed arms over the chest, anterior/lateral inclination, and static stretches; 3) exercises focused on perception and cognition (such as double-task), attention, memory, and processing of requested actions (25 min), such as walking and naming fruit/color names, completing previously established circuits, attending to requested verbal commands, and memorizing motor/verbal signals; and 4) stretching, breathing, and relaxation movements to cool down (5 min).

The sessions aimed to develop a safe and progressive training schedule and the level of difficulty of the proposed tasks increased along the program. Exercise intensity and tolerability were supervised by observation, with an emphasis on participants' respiratory responses to talk during exercise performance. In cases where the exercise intensity was not perceived as tolerable by the participants, the intensity was reduced until being perceived as comfortable (Marmeleira et al., 2018).

Blood Samples and Cytokine Measurement

Fasting venous blood samples were collected from the antecubital vein into EDTA tubes (8 ml), centrifuged (1,000 *g*, 10 min), aliquoted into microtubes, and stored at -80°C. Blood collection was performed before the first exercise session and 48 h after the last exercise bout. We selected a panel of cytokines related to metabolic, endocrine, and immunological effects that are impacted by aging. The systemic levels of interleukin (IL)-1ra, IL-1β, IL-2, IL-6, IL-10, IL-17, interferon (IFN)-α, tumor necrosis factor (TNF)-α (all from Thermo Fisher, United States), fibroblast growth factor (FGF)-1, platelet-derived growth factor (PDGF), and transforming growth factor (TGF)-α (all from RayBiotech, United States) were determined by enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions in a microplate reader (EzReader, Biochrom, United States). The coefficients of variation of all assays were always <7.5%.

Cell Culture Experiments

The PC3 prostate cancer cell line from the American Type Culture Collection (ATCC® CRL-1435™) was used in this study. PC3 cells were used in this study due to their use in investigating biochemical changes in advanced prostate cancer cells and their characteristic of androgen unresponsiveness which makes them ideal for aging studies. Cells were cultured in 75 cm^2 flasks using Roswell Park Memorial Institute medium-1640 (RPMI-1640) supplemented with 10% (v/v) fetal bovine serum (FBS), 0.1 mg/ml streptomycin, and 100 U/mL penicillin. Cells were maintained in a humidified incubator at 37°C and at 5% CO_2, during a maximum of 15 passages. Cells were plated in a 96-well plate with 10% FBS for 24 h before replacing the FBS with human plasma. During experiments, 10% FBS was replaced with 10% of human plasma obtained from older adults before or after the training sessions and from young lean men with low and high PF. Plasma obtained before and after training was incubated separately with PC3 cells. All experiments were done in triplicate, and the results were shown as the mean of triplicates from three independent experiments.

After a 48-h treatment, the cell viability assay [3-(4,5-dimethylthiazole bromide-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and LDH release] was performed by colorimetric reduction of MTT to formazan. Samples were read using a spectrophotometer at 492 nm (Mosmann, 1983). Appropriate controls with dimethyl sulfoxide (DMSO) (extract solvent) and the blank liposome were performed to eliminate the membrane solvent hydrolysis effect in the interpretation of the results. PC3 cells treated with pre-post-training plasma obtained from older adults were also incubated with N-acetylcysteine (NAC, 2 mM) for 24 h before the cell viability determination by MTT.

LDH activity was evaluated in a commercial kit (LDH Roche, Brazil). Released LDH in the culture media was coupled to an enzymatic assay yielding a red color, the intensity of which was measured at 490 nm using a microplate reader.

The proliferative response of PC3 was evaluated by the decay of carbofluorescein succinimidyl ester (CFSE) fluorescence using an FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer equipped with a blue argon laser (488 nm) and a 530/30 nm bandpass filter. The CFSE fluorescence was analyzed in histograms of the FL1 channel. The "M1 region" was defined as CFSE-stained cells derived from unstimulated cultures, which represented the peak of quiescent cells, and the M2 region was defined as proliferative cells according to the peaks of CFSE intensity. Apoptosis and necrosis of PC3 cells were measured using FITC Annexin V with a propidium iodide Apoptosis/necrosis Detection Kit (556547, BD Biosciences) according to the manufacturer's instructions using an FACSCalibur flow cytometer (BD Biosciences).

Mitochondrial membrane polarization and cytosolic and mitochondrial reactive oxygen species (ROS) analyses were performed after 12 h of *in vitro* PC3 incubation with pre- and post-exercise training plasma. The mitochondrial membrane potential ($\Delta\Psi m$) was quantified according to a method previously described (Ferlini and Scambia, 2007) using the fluorescent dye rhodamine 123 (Rh 123, Sigma-Aldrich, United States). Mitochondrial superoxide generation in live cells was assessed

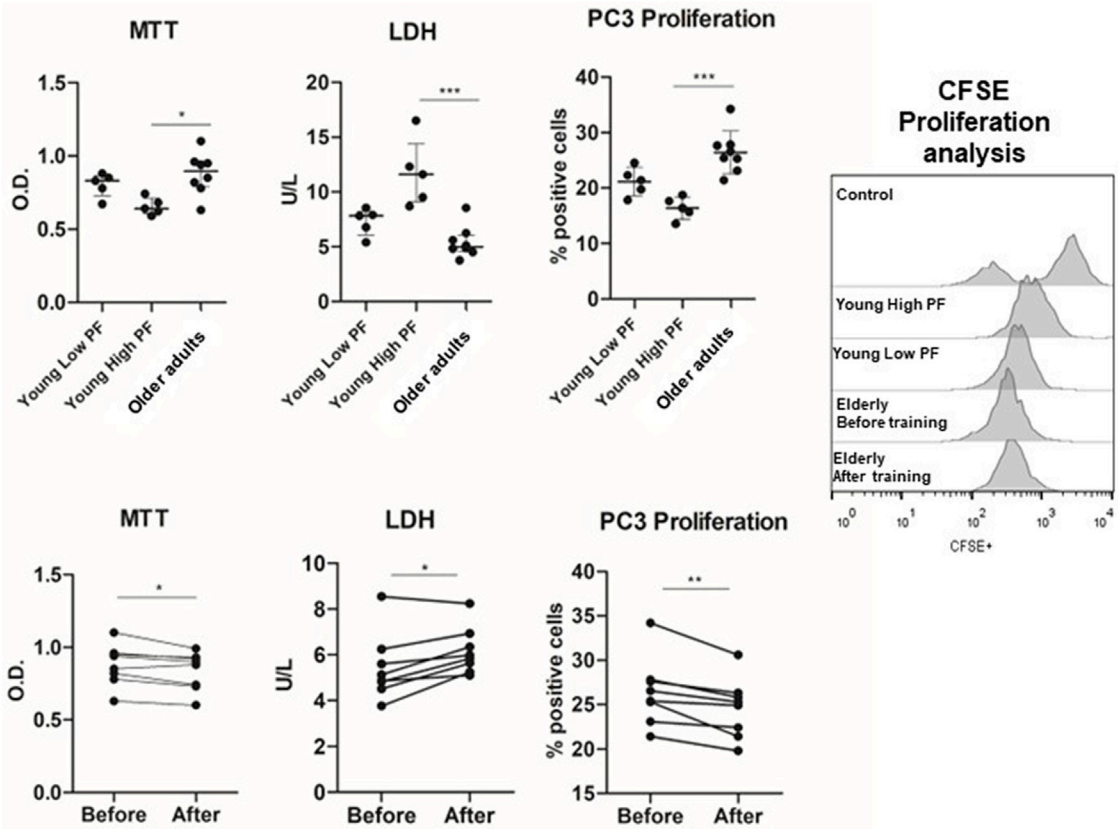

FIGURE 1 | Effects of plasma from young high-PF adults (n = 5), young low-PF adults (n = 5), and older adults (n = 8) submitted to multimodal exercise training on viability and proliferation of the PC3 prostate cancer cell line. PC3 prostate cancer cell lines were incubated with the plasma of young high-PF adults and young low-PF adults and with the plasma of institutionalized older adults obtained before training and 48 h after 8 weeks of exercise training. Cell viability was evaluated by MTT and LDH activity and proliferation by the drop of CFSE fluorescence in PC-3 cells after 48 h of cell culture. All experiments were done in triplicate, and the results are shown as the mean of triplicates from three independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001.

with MitoSOX Red (Invitrogen, Thermo Fisher, United States). Cytosolic ROS production was evaluated using the reagent 20,70-dichlorofluorescein diacetate (DCF-DA), which becomes fluorescent when oxidated by ROS (Sigma Aldrich, United States). Analyses were performed by using CELLQuest Pro Software (BD Bioscience) on an FACSCalibur flow cytometer (BD Bioscience).

Statistical Analysis

Data were analyzed in GraphPad Prism 8.0 (United States). Data were presented as mean ± standard deviation. Nonparametric tests were used in this study because of the small sample size of *in vitro* experiments. The before–after exercise training comparisons were analyzed by the Wilcoxon matched-pairs signed rank test. The effects of plasma obtained from high PF and low PF as well as the plasma collected before and after exercise training from older adults on cell viability evaluated by the MTT assay, LDH release, and PC3 cell proliferation were compared by the Kruskal–Wallis test corrected by Dunn's post-test for multiple comparisons. Spearman's correlation test was applied to verify the relationship between systemic inflammatory mediators and *in vitro* PC3 measurements. p-value ≤0.05 was considered statistically significant.

RESULTS

Prostate Cancer Cell Viability and Proliferation Are Modulated by Physical Fitness Status and Exercise Training Practice

All participants completed all intervention trial sessions, and no adverse effect was reported. Furthermore, all participants provide blood samples before and after exercise training. First, we evaluated the effects of plasma obtained from young individuals with different PF statuses (low and high) and from older adults before the engagement in a multimodal exercise training (**Figures 1**, **2**). PC3 incubated with plasma acquired from young men with high PF presented low cell viability (p = 0.01) and higher LDH released (p = 0.001) compared to the incubation trial with plasma obtained from the older adults. The percentage of cells undergoing proliferation was lower in the incubation of PC3 with plasma from the high-PF group compared to the older adults (p = 0.001). However, the analyses of PC3 cell viability or proliferation in the low-PF young group did not differ compared to the other groups (p > 0.05).

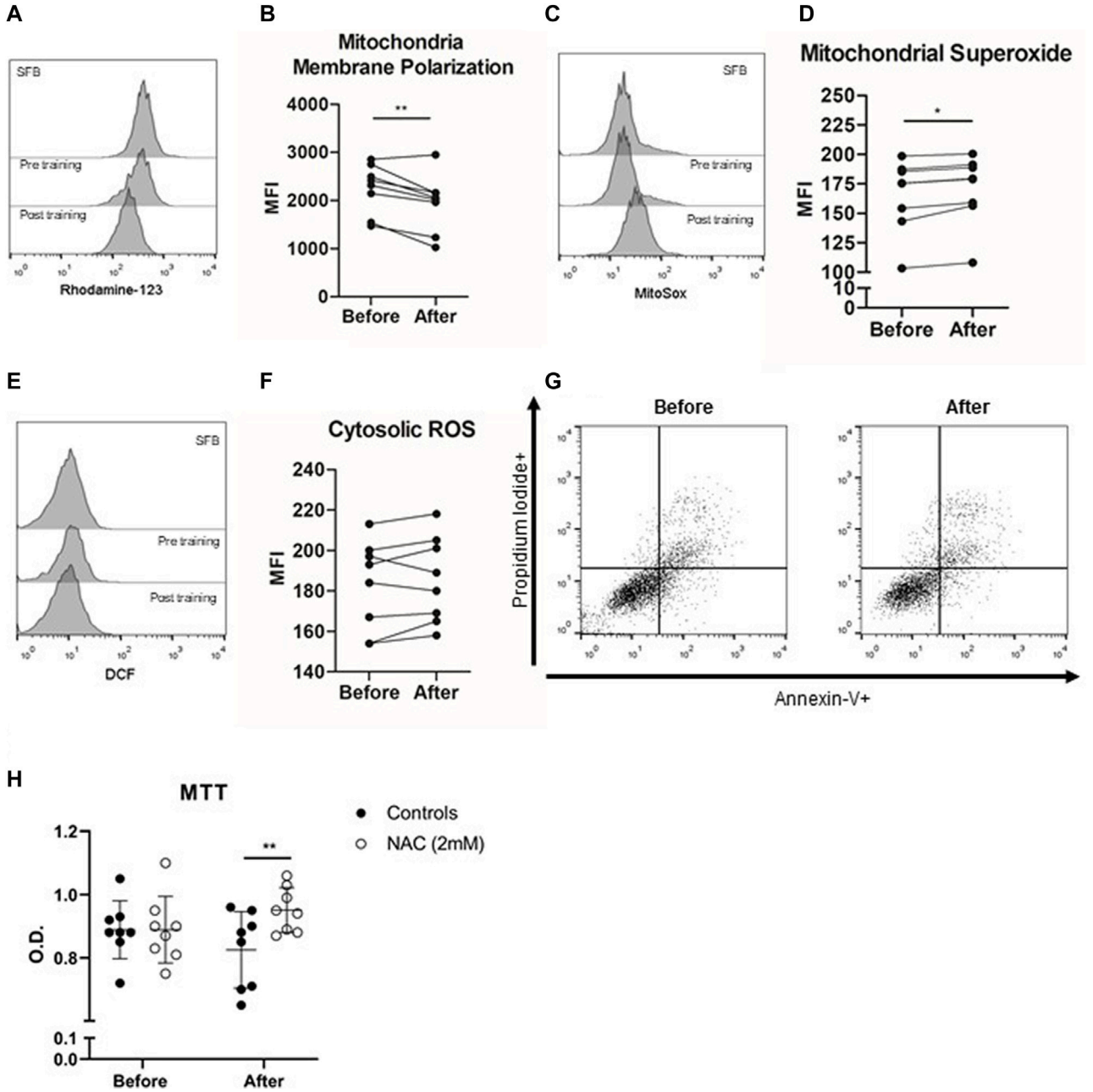

FIGURE 2 | Anticarcinogenic effects of plasma from trained older adults (n = 8) in the PC3 prostate cancer cell line. PC-3 Prostate cancer cell lines were incubated with the plasma of institutionalized older adults obtained before training and 48 h after 8 weeks of exercise training. The histogram of rhodamine-123 **(A)** evaluated the mitochondrial membrane potential **(B)**, the histogram of Mitosox **(C)** quantified mitochondrial superoxide **(D)** generation, and the histogram of DCF **(E)** evaluated cytosolic ROS **(F)** production after 12 h of PC3 cell line incubation with plasma obtained before and after exercise training. Apoptosis (Annexin-V+ cells) or necrosis (propidium iodide + cells) did not differ before and after comparison **(G)**. **(H)** Cell viability (MTT) was evaluated without and with NAC concomitant with the incubation of PC3 cells with plasma from older adults. All experiments were done in triplicate, and the results are shown as the mean of triplicates from three independent experiments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Thus, plasma factors in the blood of individuals with a higher PF status presented anticarcinogenic effects in a prostate cancer cell line compared to those observed in the plasma of older adults. Next, older adults were submitted to 8 weeks of multimodal exercise training, and plasma was obtained after the intervention. Interestingly, the incubation of the PC3 prostate cancer cell line with the post-training plasma of older adults resulted in lower cell viability (p = 0.03) and cell proliferation (p = 0.007) and higher LDH release (p = 0.02) compared to the pre-training plasma condition.

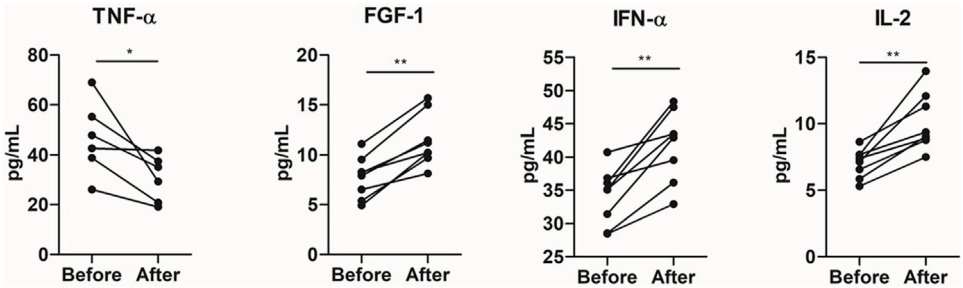

FIGURE 3 | Impact of 8 weeks of multimodal exercise training on systemic cytokine levels of institutionalized older adults. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Plasma Acquired After Multimodal Exercise Training Changes the Mitochondria Membrane Potential and Increases Mitochondrial Reactive Oxygen Species Production

Next, we evaluated the role of mitochondria-induced ROS generation in the anticarcinogenic effects of plasma of trained older adults. After 12 h of PC3 cell culture, post-training plasma decreased mitochondrial membrane polarization compared to pre-training plasma incubation conditions ($p < 0.01$). The mitochondrial membrane depolarization was accompanied by increased mitochondrial ROS production in the PC3 cell line incubated with post-training plasma ($p = 0.007$), without changes in cytosolic ROS generation ($p > 0.05$). Finally, PC3 cell line incubation with post-training plasma did not change apoptosis or necrosis cell events ($p > 0.05$). In addition, we treated PC-3 prostate cancer cells with NAC, an antioxidant molecule and a glutathione/trypanothione precursor, to evaluate if the viability of PC3 cells could be preserved. When we added NAC in the exercised plasma treatment of PC3 cells, the action of conditioned plasma was almost completely reversed ($p = 0.038$).

Multimodal Exercise Training Alters Systemic Cytokine Levels of Institutionalized Older Adults

Increases in the plasma levels of interleukin (IL)-2 ($p = 0.007$), interferon-alpha (IFN-α) ($p = 0.007$), and fibroblast growth factor (FGF)-1 ($p = 0.001$) occurred after the training period. On the other hand, tumor necrosis factor-alpha (TNF-α) concentrations decreased ($p = 0.03$) after training (**Figure 3**). No statistical differences were observed in IL-1ra, IL-1β, IL-6, IL-10, PDGF, IL-17a, or TGF-α ($p > 0.05$) (**Supplementary Figure S2**).

The percentage (%) of change of cytokine plasma levels in response to multimodal exercise training was correlated with the percentage of changes of *in vitro* experiments, MTT, LDH, CFSE cell proliferation, cytosolic ROS, mitochondrial membrane polarization, and mitochondrial ROS, in the PC3 prostate cancer cell line. IL-10 correlated with mitochondrial membrane polarization ($r = 0.82$; $p = 0.04$), TNF-α inversely correlated with mitochondrial ROS ($r = -0.94$; $p = 0.005$), IL-6 positively correlated with mitochondrial ROS ($r = 0.82$; $p = 0.04$), and cell viability evaluated by MTT correlated with IL-17 ($r = 0.76$; $p = 0.02$).

DISCUSSION

The main results of this study were as follows: 1) the incubation of the PC3 prostate cancer cell line with plasma acquired from young men with high PF leads to lower cell viability and proliferation compared to the cell treatment with pre-training plasma obtained from older adults; 2) post-exercise training plasma of older adults leads to lower cell viability and proliferation rates in PC3 prostate cancer cells; 3) conditioned post-training plasma induced mitochondrial membrane depolarization and higher mitochondrial ROS, but not cytosolic ROS, in PC3 prostate cancer cells without changes in apoptosis/necrosis rate; 4) 8 weeks of multimodal exercise training increases the systemic levels of IL-2, IFN-α, and FGF-1 and decreases the TNF-α concentrations in aged individuals. Taken together, we showed for the first time that multimodal exercise training induces systemic inflammatory adaptations in institutionalized older adults in parallel to the enhanced anticarcinogenic potential of blood mediators against prostate cancer through changes in mitochondrial membrane polarization and mitochondrial ROS generation.

The plasma collected from highly conditioned young men, but not from low-PF individuals, decreased the PC3 cell viability and lowered cell proliferation. These results are in line with the results previously reported by a series of systematic reviews and meta-analysis recently published, which demonstrated the anticarcinogenic potential of peripheral blood factors of exercised individuals (Metcalfe et al., 2021; Soares et al., 2021). The regular practice of exercise leads to chronic adaptations that reduce cancer risk through changes in cancer risk circulating biomarkers (Friedenreich et al., 2017). The difference between young and older adults might also occur due to the age-related changes in immune functions that contribute to the relationship concerning cancer and the aging process (Ames and Gold, 1991). The mechanisms of cancer development with age involve the chronic elevations in concentrations of pro-inflammatory cytokines resulting in low-grade inflammation together with a lifetime accumulation of DNA mutation, leading to inevitable errors during repair or replication of damaged DNA (López-Otín

et al., 2013; Ferrucci and Fabbri, 2018). Notwithstanding, the present data reinforce the concept that plasma from young trained human participants can modulate cancer cell viability and proliferation and can be applied to probe mechanisms and countermeasures to age-related cancer development.

Interestingly, apoptosis and necrosis rates were unchanged after the incubation of the PC3 prostate cell line with conditioned exercised plasma, confirming previous data that demonstrate that exercised mediators reduce cell viability without changes in cell death pathways (Rundqvist et al., 2013; Matos et al., 2021). Notably, several studies were conducted using acute exercise session models, and the tumor-suppressive effects of chronic exercise training are poorly studied. Moreover, some past longitudinal studies contrast with our findings regarding the role of plasma collected after exercise training on changes in cancer cell viability and proliferation (Devin et al., 2019; Soares et al., 2021). In a recent systematic review with meta-analysis, Soares and others (2021) identified that the viability of cancer cells did not change after post-training plasma stimuli. The authors indicated that conflicting results may be related to the population study, cancer cell lineage, or exercise intervention types. On the other hand, prostate cancer cells may be more susceptible to changes in post-training plasma composition. In this line, plasma acquired from trained rats reduced prostate cancer cell viability without changes in other key prostate tumor characteristics (i.e., migration and cell cycle) (Opoku-Acheampong et al., 2019). Similarly, two recent studies from the same research group demonstrated that long-term supervised multimodal exercise training changes blood factor composition and the incubation of cancer cells with the trained serum reduces tumor cell growth (Kim J.-S. et al., 2022; Kim JS. et al., 2022).

Here, we describe for the first time the mitochondrial dysfunction in the PC3 prostate cell line incubated with post-exercise training plasma of older adults. Targeting cancer cell mitochondria has been long suggested as a therapeutic approach to control cell proliferation and growth (Chattopadhyay and Roy, 2017). In this sense, several pharmacological therapies alter mitochondrial functions to induce cell death and lower tumor progression (Wen et al., 2013). In the present study, we show that depolarization of the mitochondrial membrane potential is associated with increasing superoxide production (mitochondrial ROS) after 12 h of PC3 incubation with the conditioned plasma of the older adults. Furthermore, mitochondrial membrane depolarization leads to translocation of apoptosis-induced factor (AIF) to the nuclei and activation of caspase-12 associated with the endoplasmic reticulum to induce cell death (Wang and Youle, 2009). Moreover, mitochondrial membrane depolarization directly affects complex II and its function in electric chain transport, leading to ROS generation and the activation of the apoptotic cascade (Zorov et al., 2014). Furthermore, increases in p53 protein expression in prostate tumor cells by exercise conditioned serum were previously related to a reduction in cell growth and proliferation (Leung et al., 2004). P53 is commonly described as a tumor suppressor gene by its role in conserving genome stability and preventing DNA mutation and becomes activated in response to myriad stressors, including oxidative stress (Leung et al., 2004). However, the lack of changes in apoptosis rate after conditioned plasma incubation may indicate the need for repeated or prolonged incubation time to induce cancer cell death. Notably, decreases in mitochondria membrane depolarization result in elevated cytochrome c release, a marker of low cell viability, since proper levels of cellular ATP and redox balance are essential for cell viability and proliferation (MacDonald et al., 2018). Furthermore, pharmacological suppression of NADPH oxidase (NOX), an enzymatic complex capable of oxidizing NAPH or NADH to NADP+ or NAD+, directly impacts cancer cell mitochondria, leading to a decrease in cellular glycolysis and a loss of cell viability and growth (Lu et al., 2012). Furthermore, we cannot exclude the fact that mitochondrial membrane depolarization and ROS generation may directly impact on cell viability and proliferation by targeting the Warburg effect, an event related to increases in aerobic glycolysis that supports tumor growth (Wen et al., 2013).

Here, exercise training was able to decrease pro-inflammatory TNF-α levels, suggesting a role to induce an anti-inflammatory profile in institutionalized elderlies. However, other classic proinflammatory mediators, such as IL-6, IL-17a, and IL-1β, did not change after 8 weeks of multimodal exercise. These results may suggest that the potential anti-inflammatory adaptations observed in previous observational and exercise training studies (Sellami et al., 2021) may need a longer intervention time than 8 weeks to be achieved. On the other hand, this is the first study to observe increased FGF-1 increased after an exercise training period. FGF-1, also called acidic FGF, plays an important role in the regulation of cell survival, cell division, angiogenesis, cell differentiation, and migration (Li, 2018). Interestingly, experimental studies show that mice treated with FGF-1 restore blood glucose levels and endothelial functions, highlighting the role of this growth factor in vascular health and metabolic control (Keeley et al., 2019). Furthermore, the mutated *fgf1* gene is linked to an accelerated neurological senescence profile in mice (Carter et al., 2005). Thus, FGF-1 emerges as an important biological mediator in the control of aging through exercise training.

We found an increase in IL-2 and IFN-α levels in the peripheral blood of the older adults after the exercise training period. Both IL-2 and IFN-α have strong antitumorigenic direct effects against cancer cells, and *in vitro* cytokine treatment of prostate tumor cell lines can effectively alter a number of prostate carcinoma properties closely associated with tumor invasion and the metastatic phenotype (Westdorp et al., 2014). IFN has an important role in the regulation of mitochondrial functions, and seminal studies pointed out that IFN treatment causes a reduction in cellular ATP levels and inhibits tumor growth (Shan, Vazquez, Lewis, 1990; Lewis et al., 1996). Furthermore, IFN type I has a cross-over interaction with mitochondrial ROS to control cell proliferation and survival (Yim et al., 2012; Wang et al., 2017). The correlation between post-training cytokine levels and PC3 cell viability, proliferation, and mitochondrial functions revealed some associations between changes in systemic inflammatory mediators and the cancer cell phenotype. In this line, a recent study conducted by Orange et al. (2022) indicated that systemic myokine release induced by exercise directly impacts tumor cell DNA damage induction and repair which may be associated with reductions in cancer cell proliferation.

In conclusion, this exercise training study described for the first time the potential of conditioned plasma to decrease cell viability and proliferation in the PC3 prostate tumor cell line. We also

demonstrated a new mechanistic pathway by which exercise may alter prostate cell functions through mitochondrial functions, mainly by mitochondrial membrane depolarization and superoxide formation. These changes were accompanied by alterations in several systemic inflammatory mediators after multimodal exercise training. Collectively, changes in blood factor composition by exercise training contribute to the control of prostate tumorigenesis, suggesting the role of exercise as an adjuvant therapy in cancer treatment and prevention.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Research Committee of Centro Universitario Metodista-IPA. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Credit author statement: AP designated and coordinated the study, analyzed and discussed the data, and reviewed the manuscript. GD performed the flow cytometry experiments, analyzed experimental data, and wrote the manuscript. GB collected the blood, performed the flow cytometry experiments, and drafted the manuscript. BM performed the flow cytometry experiments, analyzed experimental data, and wrote the manuscript. FN collected the blood, performed the flow cytometry experiments, and drafted the manuscript. PR designed and coordinated the study, analyzed and discussed the data, and wrote the manuscript. LM supervised the exercise training, performed the cytokine evaluation, analyzed experimental data, and drafted the manuscript. MC performed the cytokine and ROS evaluations and drafted the manuscript. VE analyzed and discussed the data and reviewed the manuscript. FL supervised the exercise training, performed the cytokine and ROS evaluations, and drafted the manuscript. TO-O performed *in vitro* experiments, drafted the manuscript and reviewed the final study version.

FUNDING

We are grateful to the Brazilian agencies Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)—Finance Code 001 for financial support. GD is supported by a post-doctoral fellowship from CAPES. PR, FL, and AP are grateful to CNPq for the PQ productivity scholarship. The authors thank UNESP for the financial support.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2022.855133/full#supplementary-material

REFERENCES

Ames, B. N., and Gold, L. S. (1991). Endogenous Mutagens and the Causes of Aging and Cancer. *Mutat. Res.* 250 (1–2), 3–16. doi:10.1016/0027-5107(91)90157-J

Barbé-Tuana, F., Funchal, G., Schmitz, C. R. R., Maurmann, R. M., and Bauer, M. E. (2020). The Interplay between Immunosenescence and Age-Related Diseases. *Semin. Immunopathol.* 42 (5), 545–557. doi:10.1007/S00281-020-00806-Z

Bauer, M. E., and De la Fuente, M. (2014). Oxidative Stress, Inflammaging, and Immunosenescence. *Inflamm. Adv. Age Nutr. Res. Clin. Interventions* 2014, 39–47. doi:10.1016/B978-0-12-397803-5.00004-6

Boland, M. L., Chourasia, A. H., and Macleod, K. F. (2013). Mitochondrial Dysfunction in Cancer. *Front. Oncol.* 3, 292. doi:10.3389/FONC.2013.00292

Carter, T. A., Greenhall, J. A., Yoshida, S., Fuchs, S., Helton, R., Swaroop, A., et al. (2005). Mechanisms of Aging in Senescence-Accelerated Mice. *Genome Biol.* 6 (6), R48. doi:10.1186/GB-2005-6-6-R48

Chattopadhyay, E., and Roy, B. (2017). Altered Mitochondrial Signalling and Metabolism in Cancer. *Front. Oncol.* 7, 43. doi:10.3389/fonc.2017.00043

Devin, J. L., Hill, M. M., Mourtzakis, M., Quadrilatero, J., Jenkins, D. G., and Skinner, T. L. (2019). Acute High Intensity Interval Exercise Reduces Colon Cancer Cell Growth. *J. Physiol.* 597, 2177–2184. doi:10.1113/JP277648

Dorneles, G. P., Silva, I., Boeira, M. C., Valentini, D., Fonseca, S. G., Dal Lago, P., et al. (2019). Cardiorespiratory Fitness Modulates the Proportions of Monocytes and T Helper Subsets in Lean and Obese Men. *Scand. J. Med. Sci. Sports* 29, 1755–1765. doi:10.1111/sms.13506

Ferlini, C., and Scambia, G. (2007). Assay for Apoptosis Using the Mitochondrial Probes, Rhodamine123 and 10-N-Nonyl Acridine Orange. *Nat. Protoc.* 2, 3111–3114. doi:10.1038/nprot.2007.397

Ferrucci, L., and Fabbri, E. (2018). Inflammageing: Chronic Inflammation in Ageing, Cardiovascular Disease, and Frailty. *Nat. Rev. CardiolCardiology* 15 (9), 505–522. doi:10.1038/S41569-018-0064-2

Fraga, I., Elsner, V., Weber, C., Galiano, W., Iraci, L., Wohlgemuth, M., et al. (2021). Effects of a Multimodal Exercise Protocol on Functional Outcomes, Epigenetic Modulation and Brain-Derived Neurotrophic Factor Levels in Institutionalized Older Adults: A Quasi-Experimental Pilot Study. *Neural Regen. Res.* 16 (12), 2479–2485. doi:10.4103/1673-5374.313067

Friedenreich, C. M., Shaw, E., Neilson, H. K., and Brenner., D. R. (2017). Epidemiology and Biology of Physical Activity and Cancer Recurrence. *J. Mol. Med.* 95 (10), 1029–1041. doi:10.1007/S00109-017-1558-9

Hwang, J. H., McGovern, J., Minett, G. M., Della Gatta, P. A., Roberts, L., Harris, J. M., et al. (2020). Mobilizing Serum Factors and Immune Cells through Exercise to Counteract Age-Related Changes in Cancer Risk. *Exerc Immunol. Rev.* 26, 80–99.

Jana, S., Patra, K., Sarkar, S., Jana, J., Mukherjee, G., Bhattacharjee, S., et al. (2014). Antitumorigenic Potential of Linalool Is Accompanied by Modulation of Oxidative Stress: An *In Vivo* Study in Sarcoma-180 Solid Tumor Model. *Nutr. Cancer* 66 (5), 835–848. doi:10.1080/01635581.2014.904906

Keeley, T., Kirov, A., Koh, W. Y., Demambro, V., Bergquist, I., Cotter, J., et al. (2019). Resistance to Visceral Obesity Is Associated with Increased Locomotion in Mice Expressing an Endothelial Cell-specific Fibroblast Growth Factor 1 Transgene. *Physiol. Rep.* 7 (7), e14034. doi:10.14814/PHY2.14034

Kim, J. S., Taaffe, D. R., Galvão, D. A., Hart, N. H., Gray, E., Ryan, C. J., et al. (2022b). Exercise in Advanced Prostate Cancer Elevates Myokine Levels and

Suppresses Iin-Vvitro Cell Growth. *Prostate Cancer Prostatic Dis.* 25 (1), 86–92. doi:10.1038/s41391-022-00504-x

Kim, J.-S., Galvão, D. A., Newton, R. U., Gray, E., and Taaffe, D. R. (2021). Exercise-Induced Myokines and Their Effect on Prostate Cancer. *Nat. Rev. Urol.* 18, 519–542. doi:10.1038/S41585-021-00476-Y

Kim, J.-S., Wilson, R. L., Taaffe, D. R., Galvão, D. A., Gray, E., and Newton, R. U. (2022a). Myokine Expression and Tumor-Suppressive Effect of Serum after 12 Wk of Exercise in Prostate Cancer Patients on ADT. *Med. Sci. Sports Exerc* 54, 197–205. doi:10.1249/MSS.0000000000002783

Leung, P.-S., Aronson, W. J., Ngo, T. H., Golding, L. A., and Barnard, R. J. (2004). Exercise Alters the IGF axis *In Vivo* and Increases P53 Protein in Prostate Tumor Cells *In Vitro*. *J. Appl. Physiology* 96, 450–454. doi:10.1152/japplphysiol.00871.2003

Lewis, J. A., Huq, A., and Najarro, P. (1996). Inhibition of Mitochondrial Function by Interferon. *J. Biol. Chem.* 271, 13184–13190. doi:10.1074/jbc.271.22.13184

Li, X. (2018). FGFs in Injury Repair and Regeneration. *Fibroblast Growth Factors* 2018, 17–144. doi:10.1016/B978-0-12-816142-5.00002-3

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., and Kroemer, G. (2013). The Hallmarks of Aging. *Cell* 153 (6), 1194–1217. doi:10.1016/J.CELL.2013.05.039

Lu, W., Hu, Y., Chen, G., Chen, Z., Zhang, H., Wang, F., et al. (2012). Novel Role of NOX in Supporting Aerobic Glycolysis in Cancer Cells with Mitochondrial Dysfunction and as a Potential Target for Cancer Therapy. *PLoS Biol.* 10 (5), e1001326. doi:10.1371/journal.pbio.1001326

MacDonald, J. A., Kura, N., Sussman, C., and Woods, D. C. (2018). Mitochondrial Membrane Depolarization Enhances TRAIL-Induced Cell Death in Adult Human Granulosa Tumor Cells, KGN, through Inhibition of BIRC5. *J. Ovarian Res.* 11, 89. doi:10.1186/s13048-018-0463-3

Marmeleira, J., Galhardas, L., and Raimundo, A. (2018). Exercise Merging Physical and Cognitive Stimulation Improves Physical Fitness and Cognitive Functioning in Older Nursing Home Residents: a Pilot Study. *Geriatr. Nurs.* 39, 303–309. doi:10.1016/j.gerinurse.2017.10.015

Matos, B., Patrício, D., Henriques, M. C., Freitas, M. J., Vitorino, R., Duarte, I. F., et al. (2021). Chronic Exercise Training Attenuates Prostate Cancer-Induced Molecular Remodelling in the Testis. *Cell Oncol.* 44 (2), 311–327. doi:10.1007/S13402-020-00567-9

Metcalfe, R. S., Kemp, R., Heffernan, S. M., Churm, R., Chen, Y. C., Ruffino, J. S., et al. (2021). Anti-Carcinogenic Effects of Exercise-Conditioned Human Serum: Evidence, Relevance and Opportunities. *Eur. J. Appl. Physiology* 121, 8. doi:10.1007/S00421-021-04680-X

Mosmann, T. (1983). Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J. Immunol. Methods* 65, 55–63. doi:10.1016/0022-1759(83)90303-4

Opoku-Acheampong, A. B., Baumfalk, D. R., Horn, A. G., Kunkel, O. N., Ganta, C. K., McCullough, D. J., et al. (2019). Prostate Cancer Cell Growth Characteristics in Serum and Prostate-Conditioned Media from Moderate-Intensity Exercise-Trained Healthy and Tumor-Bearing Rats. *Am. J. Cancer Res.* 9, 650–667.

Orange, S. T., Jordan, A. R., Odell, A., Kavanagh, O., Hicks, K. M., Eaglen, T., et al. (2022). Acute Aerobic Exercise-conditioned Serum Reduces Colon Cancer Cell Proliferation *In Vitro* through Interleukin-6-induced Regulation of DNA Damage. *Intl J. Cancer* 151 (2), 265–274. doi:10.1002/ijc.33982

Orange, S. T., Jordan, A. R., and Saxton, J. M. (2020). The Serological Responses to Acute Exercise in Humans Reduce Cancer Cell Growth *In Vitro*: A Systematic Review and Meta-analysis. *Physiol. Rep.* 8, 22. doi:10.14814/PHY2.14635

Pereira, C., Rosado, H., Cruz-Ferreira, A., and Marmeleira, J. (2018). Effects of a 10-Week Multimodal Exercise Program on Physical and Cognitive Function of Nursing Home Residents: A Psychomotor Intervention Pilot Study. *Aging Clin. Exp. Res.* 30 (5), 471–479. doi:10.1007/S40520-017-0803-Y

Rundqvist, H., Augsten, M., Strömberg, A., Rullman, E., Mijwel, S., Kharaziha, P., et al. (2013). Effect of Acute Exercise on Prostate Cancer Cell Growth. *PloS One* 8 (7), e67579. doi:10.1371/JOURNAL.PONE.0067579

Sellami, M., Bragazzi, N. L., Aboghaba, B., and Elrayess, M. A. (2021). The Impact of Acute and Chronic Exercise on Immunoglobulins and Cytokines in Elderly: Insights from a Critical Review of the Literature. *Front. Immunol.* 12, 1873. doi:10.3389/FIMMU.2021.631873

Seo, J. H., Agarwal, E., Chae, Y. C., Lee, Y. G., Storaci, A. M., Garlick, D. S., et al. (2019). Mitochondrial Fission Factor Is a Novel Myc-dependent Regulator of Mitochondrial Permeability in Cancer. *EBioMedicine* 48, 353–363. doi:10.1016/j.ebiom.2019.09.017

Shan, B., Vazquez, E., and Lewis, J. A. (1990). Interferon Selectively Inhibits the Expression of Mitochondrial Genes: a Novel Pathway for Interferon-Mediated Responses. *EMBO J.* 9, 4307–4314. doi:10.1002/j.1460-2075.1990.tb07879.x

Soares, C. M., Teixeira, A. M., Sarmento, H., Silva, F. M., Rusenhack, M. C., Furmann, M., et al. (2021). Effect of Exercise-Conditioned Human Serum on the Viability of Cancer Cell Cultures: A Systematic Review and Meta-Analysis. *Exerc Immunol. Rev.* 27, 24–41.

Taverna, G., Seveso, M., Giusti, G., Hurle, R., Graziotti, P., Štifter, S., et al. (20142014). Senescent Remodeling of the Innate and Adaptive Immune System in the Elderly Men with Prostate Cancer. *Curr. Gerontology Geriatrics Res.* 2014, 1–11. doi:10.1155/2014/478126

Wang, C., and Youle, R. J. (2009). The Role of Mitochondria in Apoptosis. *Annu. Rev. Genet.* 43, 95–118. doi:10.1146/ANNUREV-GENET-102108-134850

Wang, Y., Yu, X., Song, H., Feng, D., Jiang, Y., Wu, S., et al. (2017). The STAT-ROS Cycle Extends IFN-induced C-ancer C-ell A-poptosis. *Int. J. Oncol.* 52, 305–313. doi:10.3892/ijo.2017.4196

Wen, S., Zhu, D., and Huang, P. (2013). Targeting Cancer Cell Mitochondria as a Therapeutic Approach. *Future Med. Chem.* 5 (1), 53–67. doi:10.4155/FMC.12.190

Westdorp, H., Sköld, A. E., Snijer, B. A., Franik, S., Mulder, S. F., Major, P. P., et al. (2014). Immunotherapy for Prostate Cancer: Lessons from Responses to Tumor-Associated Antigens. *Front. Immunol.* 5, 191. doi:10.3389/FIMMU.2014.00191

Wu, C.-L., Huang, A.-C., Yang, J.-S., Liao, C.-L., Lu, H.-F., Chou, S.-T., et al. (2011). Benzyl Isothiocyanate (BITC) and Phenethyl Isothiocyanate (PEITC)-mediated Generation of Reactive Oxygen Species Causes Cell Cycle Arrest and Induces Apoptosis via Activation of Caspase-3, Mitochondria Dysfunction and Nitric Oxide (NO) in Human Osteogenic. *J. Orthop. Res. Official Publ. Orthop. Res. Soc.* 29 (8), 1199–1209. doi:10.1002/JOR.21350

Xie, K., Huang, S., Dong, Z., Gutman, M., and Fidler, I. J. (1995). Direct Correlation between Expression of Endogenous Inducible Nitric Oxide Synthase and Regression of M5076 Reticulum Cell Sarcoma Hepatic Metastases in Mice Treated with Liposomes Containing Lipopeptide CGP 31362. *Cancer Res.* 55 (14), 3123–3131. (Accessed March 18, 2022).

Yim, H. Y., Yang, Y., Lim, J.-S., Lee, M. S., Zhang, D.-E., and Kim, K. I. (2012). The Mitochondrial Pathway and Reactive Oxygen Species Are Critical Contributors to Interferon-A/β-Mediated Apoptosis in Ubp43-Deficient Hematopoietic Cells. *Biochem. Biophysical Res. Commun.* 423, 436–440. doi:10.1016/j.bbrc.2012.05.154

Zichri, S. B., Kolusheva, S., Shames, A. I., Schneiderman, E. A., Poggio, J. L., Stein, D. E., et al. (2021). Mitochondria Membrane Transformations in Colon and Prostate Cancer and Their Biological Implications. *Biochimica Biophysica Acta (BBA) - Biomembr.* 1863 (1), 183471. doi:10.1016/J.BBAMEM.2020.183471

Zorov, D. B., Juhaszova, M., and Sollott, S. J. (2014). Magdalena Juhaszova, and Steven J. SollottMitochondrial Reactive Oxygen Species (ROS) and ROS-Induced ROS Release. *Physiol. Rev.* 94 (3), 909–950. doi:10.1152/PHYSREV.00026.2013

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TYPE Original Research
PUBLISHED 28 September 2022
DOI 10.3389/fphys.2022.838580

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SPECIALTY SECTION
This article was submitted to Exercise Physiology, a section of the journal Frontiers in Physiology

RECEIVED 18 December 2021
ACCEPTED 05 September 2022
PUBLISHED 28 September 2022

CITATION
Farinha C, Ferreira JP, Serrano J, Santos H, Oliveiros B, Silva FM, Cascante-Rusenhack M and Teixeira AM (2022), The impact of aquatic exercise programs on the systemic hematological and inflammatory markers of community dwelling elderly: A randomized controlled trial. *Front. Physiol.* 13:838580. doi: 10.3389/fphys.2022.838580

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The impact of aquatic exercise programs on the systemic hematological and inflammatory markers of community dwelling elderly: A randomized controlled trial

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Evidence shows that physical exercise is important in maintaining an efficient immune system during ageing. However, there are few studies that test the impact of aquatic exercise programs on the immune system. This study aims to analyze the impact of different physical exercise programs in aquatic environment on the systemic hematological and inflammatory markers of community dwelling elderly. One hundred and two elderly were randomly allocated into four groups: a continuous aerobic exercise group (AerG) (n = 25, 71.44 ± 4.84 years); an interval aerobic exercise group (IntG) (n = 28, 72.64 ± 5.22 years); a combined exercise group (ComG) (n = 29, 71.90 ± 5.67 years); a control group (CG) (n = 20, 73.60 ± 5.25 years). The AerG, IntG and ComG participants took part in three different aquatic exercise programs over a 28-weeks period. The CG participants maintained their usual routines during the same time period. Blood samples were collected from all participants in order to access hematologic indicators, by means of cell count, and the inflammatory profile by ELISA. After 28 weeks, significant differences were found for several hematologic variables in the AerG, IntG and ComG with increases in mean corpuscular hemoglobulin (MCH), mean corpuscular hemoglobulin concentration (MCHC), and hemoglobulin (Hb). Decreases in TNF-α levels were found for all exercising groups. An increase in IL-10 levels, granulocytes to lymphocytes ratio (GLR) and a decrease in the TNF-α/IL-10 ratio, were found for the IntG. For the ComG decreases were also found for the TNF-α, IL-1ß/IL-1ra ratios. The present study suggests that aquatic exercise programs were able to improve the inflammatory profile of the participants. Those in the exercise intervention groups showed a shift towards lower pro-inflammatory levels while the non-exercising group showed the opposite behaviour. The IntG and the ComG aquatic exercise

programs appeared to be more effective than the AerG program in decreasing chronic low-grade inflammation by mediating the production of higher levels of anti-inflammatory cytokines. However, the differences found between the exercising groups were small and may not have clinical significance.

KEYWORDS

physical exercise, aquatic environment, hydrogymnastics, ageing, interleukins, inflammageing

1 Introduction

The immune system is characterized as a complex network of cells and molecules that operate to protect the body from disease, prevent the entry of invading microorganisms and facilitate wound healing (Simpson et al., 2015).

Ageing is associated with immunosenescence, i.e., a progressive decline in the immune function, which causes changes in innate and adaptive immunity. These changes are related to increased morbidity from infectious agents, and the appearance of several age-related diseases, including cardiovascular and metabolic diseases (Sellami et al., 2018). Immunosenescence is characterized by the following aspects: decreased response to new invading infectious agents; unsupported memory T cell response; a higher susceptibility to autoimmune diseases; low-grade chronic inflammation (Rodriguez et al., 2020). A decrease in naive T cells and the consequent increase in memory T cells (Weltevrede et al., 2016) is also well documented. This reduction is considered to be a crucial factor in decreasing the ability to recognize pathogens, which increases the probability of infection (Spielmann et al., 2011). T cells are subject to continuous remodeling, that results from their interaction with different stressors from the internal and external environment. This interaction leads to the decrease of T cells, which is accompanied by a decline in the T cell receptor clonal diversity and consequent increase in the memory cell subpopulation, with an accumulation of terminally differentiated T cells that are dysfunctional or depleted (Rodriguez et al., 2020). Another important aspect of ageing is the development of sterile chronic low-grade inflammation, called inflammaging, which contributes to the pathogenesis of age-related diseases (Franceschi et al., 2018). The TNF-α/IL-10 and IL1ß/IL-1ra ratios are markers that depict the balance between important pro- and anti-inflammatory cytokines (Nisansala et al., 2021) and are associated with the development of coronary and inflammatory diseases (Kumari et al., 2018). More recently the neutrophil to lymphocyte ratio (NLR), the platelet to lymphocyte ratio (PLR) and the systemic immune-inflammation index (SII = NLR x platelets) are being used in the clinical context and provide a multifactorial view of inflammatory processes, with their implementation being increasingly common as inflammatory and prognostic markers of various pathologies such as neurological and cardiovascular diseases. (Walzik et al., 2021).

Regular physical exercise is associated with a reduction of different types of chronic diseases associated with ageing, by means of changes in immune function, specifically in the decrease of immune senescence, the increasing of the innate immune function and the decreasing of chronic inflammation (Abd El-Kader and Al-Shreef, 2018). Continuous exercise of moderate intensity and short duration is associated with an improvement in immune defense. On the other hand, high intensity exercise can cause transient negative changes in immune cell count and function, which can trigger an increased risk of infectious diseases (Souza et al., 2021). Interval exercise programs have been gaining popularity. According to the study by Souza et al. (2021), an isolated session of interval exercise can cause a transient disturbance in the immune system leading to a reduction in immune function. However, the regular practice of programs of interval exercise can induce beneficial adaptations in short- and long-term immune function without altering immune cell counts.

In contrast, a sedentary lifestyle leads to accumulation of visceral fat, which is infiltrated by pro-inflammatory macrophages and T cells. This causes pro-inflammatory macrophages to predominate and the inflamed adipose tissue to release pro-inflammatory cytokines like TNF-α, IL-1β, chemokines and increased expression of Toll like receptors (TLR), which leads to a state of meta-inflammation causing insulin resistance and metabolic disease (Kruger, 2017). In comparison, because physical exercise reduces visceral fat, it will stimulate a decrease in the production and release of adipokines, causing an anti-inflammatory environment during each exercise session (Gleeson et al., 2013). In addition to the reduction of visceral fat, physical exercise, more specifically skeletal muscle contractions, directly stimulates the production of IL6, that will produce anti-inflammatory cytokines, such as IL-10, IL-1ra, and cortisol secretion (Pedersen & Fischer, 2007).

A study by Chupel et al. (2017) concluded that a 28-weeks intervention with a muscular strength exercise program supported the increase of anti-inflammatory balance in a sample of older women. Similar results were observed by Furtado et al. (2020), after an intervention with two different

physical exercise programs (a combined exercise program, with muscle strength exercise using elastic bands and a chair-based combined exercise). In another study by Werner et al. (2019), the results showed an increase in leukocytes, granulocytes and lymphocytes after a 26-weeks intervention with aerobic exercise and interval aerobic exercise. Contrastingly, the muscle strength exercise did not produce the same increase. However, Kapasi et al. (2003) concluded that an 8-month intervention with combined exercise (aerobic exercise and muscle strength exercise) did not induce changes in lymphocyte subpopulations. Likewise, Shimizu´s et al. (2011) study showed that the number of leukocytes, lymphocytes and monocytes did not change after a 12-weeks muscle strength intervention program.

The studies mentioned above have shown that physical exercise can be a powerful tool to improve the inflammatory profile of elderly participants. However, it is still not clear which type of exercise is the most effective. Additionally, few studies have analyzed the impact of physical exercise programs within these indicators, in an aquatic environment (Bansi et al., 2013; Santos, 2020). Considering the limitations previously mentioned, the purpose of this study is to compare the impact of different physical exercise aquatic programs (continuous aerobic exercise, interval aerobic exercise and combined exercise) on the systemic hematological and inflammatory markers of community dwelling elderly, and determine which of the three aquatic exercise programs used was the most effective.

2 Materials and methods

2.1 Study design

A randomized controlled trial was conducted in the Beira Interior region, Portugal. A sample of non-institutionalized elderly participants were submitted to 28-weeks aquatic exercise intervention (October-May). The entire study protocol was previously published (Ferreira et al., 2020). To analyzed the impact of different aquatic exercise programs on the immunologic profile of elderly participants, three different physical exercise programs were used: continuous aerobic (AerG), interval aerobic exercise (IntG) and combined exercise (ComG). Blood samples were collected at two specific time moments, namely: pre-intervention (baseline, M1) and post-intervention (after 28 weeks, M2). This study was carried out according to the recommendations of the Declaration of Helsinki for Human Studies. The protocol was approved by the Ethics Committee for Health of the Faculty of Sport Sciences and Physical Education, University of Coimbra (reference: CE/FCDEF-UC/00462019). Written informed consent was obtained from all participants prior to any protocol-specific procedures.

2.2 Participants and sample size

The size and statistical power of the sample were calculated using the G*Power software application (Franz et al., 2007). The following parameters were considered: F test (ANOVA); effect size: 0.25; α-level: 0.05; statistical power: 0.95; number of groups: 4; number of measures: 2 (pre and post intervention); margin of possible losses and refusals: 30%. Therefore, the initial size of the total sample was estimated at 76 participants.

Initially, 174 individuals from the community were invited to participate in the study. After the application of the inclusion and exclusion criteria, 152 individuals were randomized into four groups: continuous aerobic exercise group (AerG, n = 36); interval aerobic exercise group (IntG, n = 41); combined exercise group (ComG, n = 48); control group (CG, n = 27). According to the experience of the research team and previous studies, the dropout rate from exercise programs among older populations is high, so we recruited more participants to compensate for potential dropouts (Dziubek et al., 2015; Chupel et al., 2017; Moreira et al., 2020). A simple randomization method was used. An external investigator used a computer-generated list of random numbers to allocate participants to each group. The investigators were blinded for the randomization of the groups.

The following inclusion criteria were applied: 1) participants from both sexes; 2) 65 years old or more; 3) non-institutionalized; 4) autonomy to move from their residence to Sertã municipal swimming pool; 5) filling out the informed consent form; 6) individuals with medical authorization to practice physical exercise in an aquatic environment, in the cases where some type of clinical condition or comorbidity was present. The following exclusion criteria were also defined: 1) individuals with clinically diagnosed pathologies putting their health and others at risk while doing physical exercise in an aquatic environment; 2) individuals that obtained a score of less than 9 points in the Mini-Mental State Examination (indicating severe cognitive impairment) or were clinically diagnosed with a mental illness.

The AerG, IntG and ComG groups performed physical exercise in an aquatic environment during the same period of 28 weeks (each group took part in a different exercise program). Participants from the CG group were asked to maintain their normal daily activities, without performing any type of systematic physical exercise during the intervention period.

Fifty participants dropped or were excluded out from the study due to the following reasons: personal reasons (n = 11); less than 50% of attendance of the exercise sessions (n = 14); did not complete all the assessment tests (n = 12); injury not related with the exercise intervention (n = 4); disease (n = 9). Consequently, 102 participants completed the entire process (AerG: n = 25, 71.44 ± 4.84 years, 80% female; IntG: n = 28, 72.64 ± 5.22 years, 89.3% female; ComG: n = 29, 71.90 ± 5.67 years, 75.9% female;

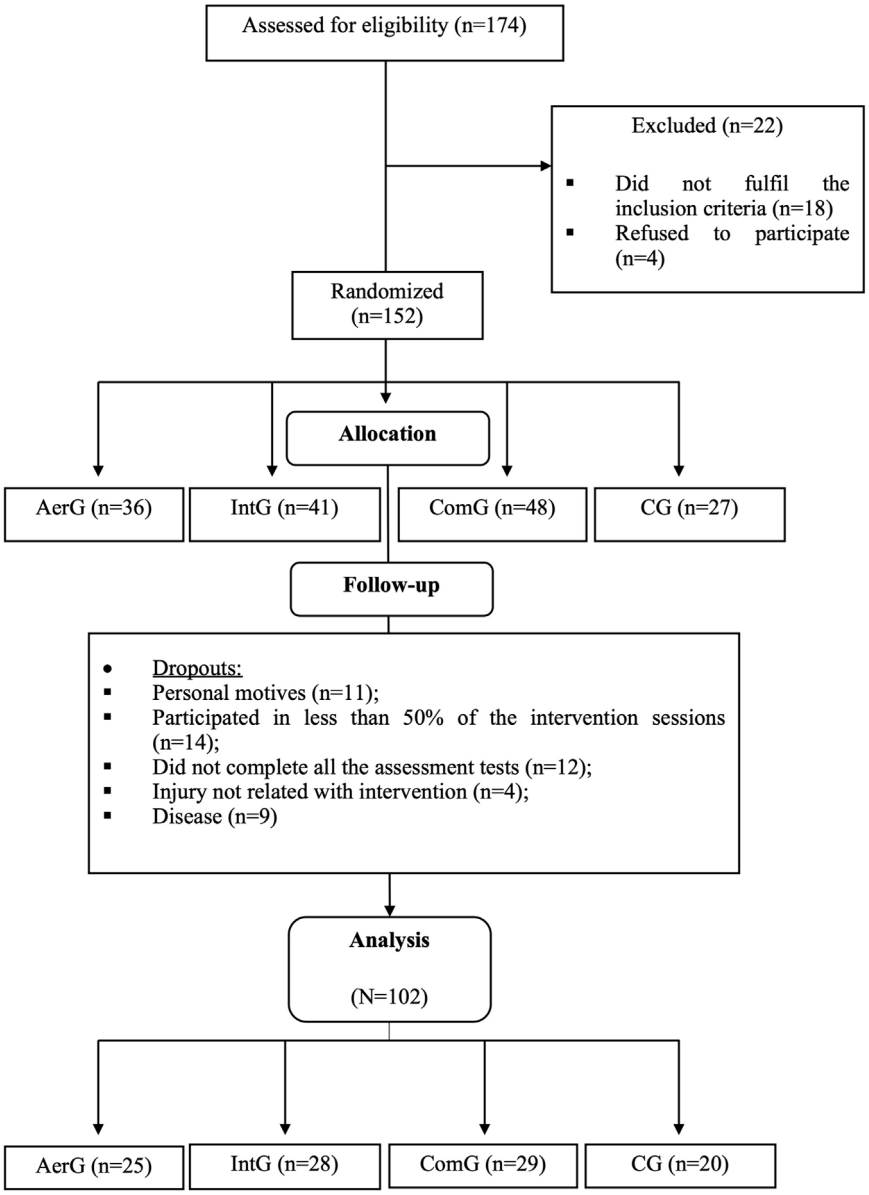

FIGURE 1
Allocation process for the different groups: Continuous aerobic group (AerG); Aerobic interval group (IntG); Combined group (ComG); Control group (CG).

CG: n = 20, 73.60 ± 5.25 years, 55% of females). Figure 1 shows the entire allocation process for the different groups.

2.3 Outcomes measurements

2.3.1 Sample characterization

Data such as age, regular medication, allergies, alcohol consumption, smoking and medication habits were also obtained in the first phase of data collection (M1) by completing a specific questionnaire. Values referring to anthropometry and functional fitness were also collected. Height (Hgt) was assessed using a portable Seca Bodymeter® stadiometer (model 208, Hamburg, Germany) with an accuracy of 0.1 cm. Weight (Wgt), body mass index (BMI), visceral fat (VF), fat mass (FM) and muscle mass (LBM) were evaluated using the TANITA BC-601 impedance scale (Tokyo, Japan). Functional fitness was assessed using the following tests from the Senior Fitness Test set, developed by Rikli and Jones (1999) and validated for the Portuguese population by Baptista and Sardinha

10.3389/fphys.2022.838580

TABLE 1 Participant's baseline characteristics by group.

	AerG (n = 25)	IntG (n = 28)	ComG (n = 29)	CG (n = 20)	p-value
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Morphological parameters					
Age (years)	71.44 (4.8)	72.64 (5.2)	71.90 (5.7)	73.60 (5.3)	0.504
Stature (m)	1.58 (0.7)	1.56 (0.7)	1.57 (0.7)	1.60 (0.9)	0.331
Body mass (kg)	70.5 (8.1)	71.3 (14.3)	75.1 (11.0)	75.5 (13.3)	0.334
BMI (kg/m^2)	28.20 (3.3)	29.10 (4.8)	30.80 (5.3)	29.50 (5.8)	0.272
VF (%)	11.0 (3.0)	12.0 (3.0)	13.0 (3.0)	13.0 (6.0)	0.128
FM (%)	38.9 (7.3)	41.0 (6,7)	40.3 (9.8)	34.9 (10.9)	0.134
LBM (%)	26.5 (4.3)	24.5 (3.0)	25.5 (4.3)	27.7 (4.7)	0.079
Physical fitness tests					
2min-ST (no of steps)	80.9 (17.4)	71.5 (16.5)	81.6 (19.2)	74.3 (18.9)	0.069
CSR-R (cm)	-0.5 (6.6)	-3.7 (10.6)	-3.5 (7.8)	-7.6 (9.7)	0.099
CSR-L (cm)	0.6 (7.2)	-3.9 (9.9)	-5.8 (9.9)	-3.5 (7.3)	0.054
BS-R (cm)	-9.9 (10.4)	-11.9 (11.5)	-14.3 (9.7)	-16.6 (9.9)	0.157
BS-L (cm)	-14.4 (7.2)	-17.4 (8.6)	-21.0 (10.8)	-20.6 (10.7)	0.056
TUG (s)	6.1 (1.1)	7.4 (1.8)	7.4 (3.0)	6.8 (1.7)	0.110
30s-CS (reps/30s)	15.0 (3.0)	13.0 (4.0)	13.0 (3.0)	15.0 (5.0)	0.185
30s-AC (reps/30s)	21.0 (6.0)	17.0 (7.0)	20.0 (5.0)	19.0 (6.0)	0.119
HG-R (kg)	22.0 (6.0)	21.0 (9.0)	21.0 (9.0)	24.0 (9.0)	0.411
HG-L (kg)	21.0 (6.0)	20.0 (9.0)	21.0 (9.0)	21.0 (10.0)	0.578
Health parameters	N (%)	N (%)	N (%)	N (%)	
Regular medication	23 (92%)	27 (96%)	26 (90%)	19 (95%)	0.768
No alcohol and smoking habits	22 (88%)	25 (89%)	26 (90%)	19 (95%)	0.772
Allergies	10 (40%)	9 (32%)	14 (48%)	7 (35%)	0.638

SD, standard deviation; AerG, continuous aerobic group; IntG, aerobic interval group; ComG, combined group; CG, control group; Hgt, height; Wgt, weight; BMI, body mass index; VF, visceral fat; FM, fat mass; LBM, muscle mass; 2min-ST, two-minute step test; CSR-R, chair sit and reach test - right; CSR-L, chair sit and reach test - left; BS-R, back scratch test-right; BS-L, back scratch test-left; TUG, timed up and go test; 30s-CS, chair stand test; HG-R, hand grip test-right; HG-L, hand grip test-left. Regular medications include: medication to control blood pressure, cholesterol and diabetes.

(2005): muscle strength of the lower (MI) and upper (MS) limbs, with the Chair Stand test (30s-CS) and Arm Curl test (30s-AC), respectively (repetitions/30s); aerobic capacity, with the Two-Minute Step test (2min-ST) (no. of steps); the flexibility of MI and MS, with the Chair Sit and Reach test (CSR) and Back Scratch test (BS), respectively (centimeters); agility and dynamic balance, with the Timed Up and Go test (TUG) (seconds). The handgrip strength was also evaluated, with the Hang Grip test (HG), using the Jamar hand dynamometer (Lafayette Instrument Company, United States) (kg). These data are presented in Table 1.

2.3.2 Biochemical markers (systemic hematological and inflammatory markers)

Participants were instructed to avoid exercise practice 72 h before blood collection. Fasting Blood samples (15 ml) were collected from the ante cubital vein by a registered nurse. A complete blood count (CBC) was conducted using an automatic hematology analyzer (Coulter Act, Beckman Coulter, United States) to obtain the values for: leukocyte (LEU); lymphocytes (LI); monocytes (MO); granulocytes (GR); erythrocytes (ERI); hemoglobulin concentration (Hb); hematocrit (Hct); mean corpuscular volume (MCV); mean globular hemoglobulin (MCH); mean corpuscular hemoglobulin concentration (MCHC); erythrocyte distribution width (RDW); platelets (PL); mean platelet volume (MPV). Next, the test tubes were centrifuged for 20 min at 800 g and stored in cryogenic test tubes at -80 °C until further use. Levels of interleukin 1 receptor antagonist (IL-1ra), interleukin 1 beta (IL-1ß), interleukin 10 (IL-10) and tumor necrosis factor (TNF-α), were subsequently analyzed by ELISA (Invitrogen®, Alfagene, Portugal) according to the manufacturer instructions.

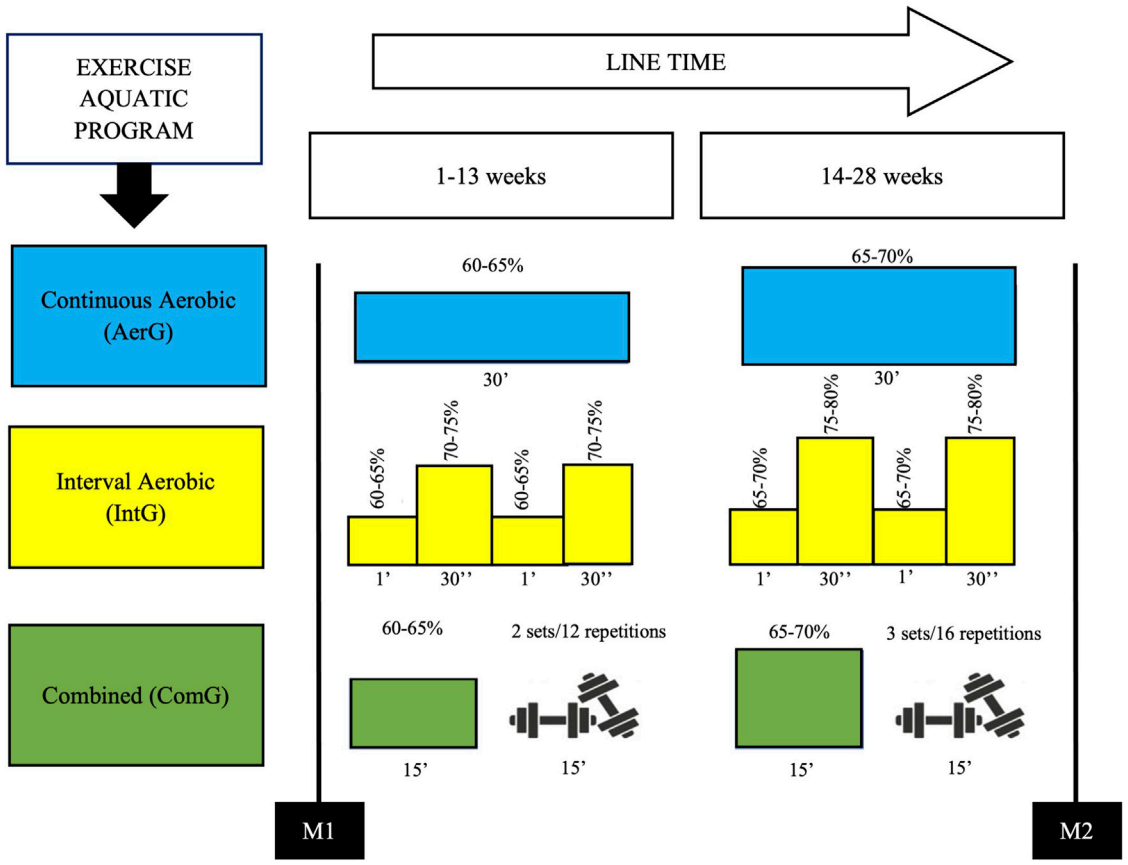

FIGURE 2
Time line, periodization and characterization of the exercise programs.

2.4 Intervention protocol

The exercise programs were implemented by Sport Sciences and fitness experts, with specific training in water aerobics and developed according to the exercise prescription guidelines recommended by the American College of Sport Medicine (ACSM) for the elderly (ACSM, 2018).

All exercise programs sessions had a duration of 45 min, twice a week, for 28 weeks and were performed in water environment (the water level was between 0.80 and 1.20 m with a temperature of approximately 32 °C), using the music rhythm as a tool to control the intensity of the exercise. Sequences of aquatic exercises, previously defined and selected according to the objectives of each program, were applied. Water exercise sessions were organized into three different parts: initial, main and final part.

The initial part or warm-up last between 10 and 15 min, at low intensity (30–40% max HR), and was the same in the three water exercise programs. This initial part, was aimed at the participants adaptation to the aquatic environment, i.e., to the water temperature, and provided muscular and metabolic stimulations to prepare the body for the main part of the session. Thus, simple exercises in water were used, such as displacements and isolated movements, with a progressive increase in complexity and intensity throughout the initial part.

The main part of the three water exercise programs sessions had a duration of 20–30 min and were characterized as follows (Figure 2):

- In the water continuous aerobic program (AerG), aerobic exercises were used continuously throughout the main part of the session (20–30 min), with a target intensity of 60–70% of the maximum heart rate, according to the recommendations of the ACSM for the elderly (ACSM, 2018);- In the water interval aerobic program (IntG), the main part of the session consisted of performing exercises with different intensities, such as: short duration exercises of 30 s, with an intensity of 70–80% of the maximal heart rate, followed by active recovery intervals of 1 min, using exercises with an intensity of 60–70% of the maximal heart rate;- In the water combined training program (ComG), the main part of the session was divided into two

phases, with equal time periods. The first phase consisted of aerobic exercises on a continuous basis, with a target intensity between 60 and 70% of the maximal heart rate. In the second phase, muscle strengthening exercises were applied (water environment): 6 to 8 different exercises, with auxiliary equipment to create more resistance to movement (e.g., "spaghetti" and dumbbells), covering muscle strengthening of trunk (e.g., rowing, inverted crunch, etc.), upper limbs (e.g., elbow flexion and extension, shoulder rotation, etc.) and lower limbs (e.g., knee flexion and extension, leg abduction and adduction, etc.). Strengthening exercises were implemented using 2 to 3 sets of 12–16 repetitions at moderate intensity (6-7 points in the Borg Scale).

The final part of the water exercise sessions lasted between 5 and 10 min and were similar for all the three water exercise programs. This part consisted of two phases: return to calm, where relaxation exercises were applied to bring back the participants' heart rate to values close to the resting state, and stretching exercises stimulating a greater range of motion, used to stretch the main muscle groups used throughout the sessions.

2.5 Monitoring the exercise programs intensity

For safety reasons and intensity target control, participants randomly used heart rate monitors (Polar, R800CX) during the three water exercise programs sessions (10 heart rate monitors were used per session). Depending on the heart rate values obtained, intensity adjustments to the training plan were performed to maintain the intensity target defined for each water exercise program.

For safety and intensity target control, the intensity of the different water exercise programs was predicted indirectly using the Karvonen´s formula (Karvonen et al., 1957):

$$Target\ heart\ rate = ((maximal\ heart\ rate - resting\ heart\ rate)\ X\ \%\ intensity) + resting\ heart\ rate$$

Additionally, and to calculate the maximal heart rate, the Franklin et al. (2000) formula for the elderly was used:

$$Maximal\ HR = 207\ beats\ per\ min. - (0.7\ X\ chronological\ age)$$

2.6 Statistical analysis

The collected data was subjected to descriptive statistical analysis where values such as maximum, minimum, mean and standard deviation were calculated for each variable in each assessment moment. Afterwards, data normality was tested by considering the response to three conditions: z-values from the Skewness and Kurtosis tests; *p*-values from the Shapiro-Wilk test; and visual inspection of generated histograms. All longitudinal comparisons were performed using complete case analysis. Parametric data was analyzed using the Student's t-test for paired samples to compare the different moments (M1 and M2) and the one-factor Anova test and post-hoc Tukey's test to analyze the differences between groups both at M1 and M2. Nonparametric data was analyzed using the Wilcoxon test to compare the different moments (M1 and M2) and the Kruskal Wallis and Bonferroni tests to analyze differences between groups. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) statistical software, version 27.0. The level of significance used was $p \leq 0.05$.

3 Results

The results from 102 non-institutionalized elderly participants at baseline, distributed into four different groups (AerG: n = 25, 71.44 ± 4.84 years; IntG: n = 28, 72.64 ± 5.22 years; ComG: n = 29, 71.90 ± 5.67 years; CG: n = 20, 73.60 ± 5.25 years) that completed a 28-weeks intervention are shown in Table 1.

Participants from all groups (AerG, IntG, ComG and CG) showed similar characteristics at baseline, with no significant statistical differences in anthropometrics, physical fitness or clinical characterization variables regarding each group.

The variation in the results for the systemic hematologic markers when analyzed by type of aquatic exercise program, before and after the 28-weeks intervention, are shown in Table 2.

No differences were found for the systemic hematologic variables between groups, before the intervention (M1). Significant differences between groups, after the intervention (M2) were found for MO# ($p = 0.013$) between the CG and the IntG participants ($p = 0.007$), as well as between the CG and the ComG ($p = 0.002$), and between the CG and the AerG ($p = 0.033$). Differences in MCHC ($p = 0.044$) between the CG and the IntG participants ($p = 0.007$) were also detected. In all identified cases, the results were lower for the CG.

As a result of the intervention, statistically significant increases were found in the following variables: LEU in the IntG ($p = 0.006$; $\Delta = 8.4\%$) and the ComG ($p = 0.044$; $\Delta = 4.4\%$); GR# in the IntG ($p = 0.006$; $\Delta = 12.1\%$) and the ComG ($p = 0.046$; $\Delta = 8.4\%$); MCH in the AerG ($p = 0.001$; $\Delta = 2.7\%$), the IntG ($p < 0.001$; $\Delta = 4.4\%$) and the ComG ($p < 0.001$; $\Delta = 4.4\%$); MCHC in the AerG ($p < 0.001$; $\Delta = 3.8\%$), the IntG ($p < 0.001$; $\Delta = 5.4\%$) and the ComG ($p < 0.001$; $\Delta = 5.4\%$); Hb in the ComG ($p = 0.038$; $\Delta = 2.2\%$); and GLR ratio in the IntG ($p < 0.046$; $\Delta = 9.6\%$). (Figure 3).

Statistically significant reductions were also found in the following variables: ERI in the AerG ($p = 0.032$; $\Delta = -2.2\%$), the IntG ($p = 0.001$; $\Delta = -2.8\%$) and the ComG ($p = 0.003$; $\Delta = -2.4\%$); Hct in the AerG ($p = 0.006$; $\Delta = -3.0\%$), the IntG ($p < 0.001$; $\Delta =$

TABLE 2 Results of systemic hematological variables at baseline and after 28 weeks of intervention.

	AerG			IntG			ComG			CG				
	M1	M2	Time (p)	M1	M2	Time (p)	M1	M2	Time (p)	M1	M2	Time (p)	Group (M1)	Group (M2)
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)			
LEU ($\times10^3$/μL)	5.99 (1.17)	6.04 (1.26)	0.838	5.85 (1.22)	6.34 (1.26)	**0.006***	6.37 (1.37)	6.65 (1.51)	**0.044****	5.70 (1.30)	5.95 (1.44)	0.183	0.269	0.335
LI (%)	35.67 (7.57)	36.28 (8.38)	0.657	34.09 (7.54)	32.76 (9.06)	0.158	35.57 (8.19)	34.00 (8.75)	0.140	37.00 (8.23)	35.17 (7.95)	0.108	0.546	0.444
MO (%)	5.76 (2.16)	5.88 (1.80)	0.844	6.39 (2.38)	6.29 (2.06)	0.809	6.57 (1.95)	6.21 (2.25)	0.542	5.99 (2.01)	5.74 (1.79)	0.665	0.505	0.741
GR (%)	58.58 (8.11)	57.84 (8.44)	0.575	59.51 (8.45)	61.23 (9.50)	0.094	57.86 (9.12)	59.79 (8.81)	0.123	57.01 (8.97)	59.23 (9.42)	0.098	0.604	0.396
LI# ($\times10^3$/μL)	2.13 (0.63)	2.17 (0.55)	0.707	2.00 (0.59)	2.07 (0.66)	0.213	2.26 (0.65)	2.22 (0.66)	0.554	2.06 (0.45)	2.04 (0.49)	0.741	0.529	0.676
MO# ($\times10^3$/μL)	0.34 (0.14)	0.36 (0.14)	0.503	0.37 (0.16)	0.39 (0.17)	0.388	0.43 (0.16)	0.41 (0.16)	0.503	0.33 (0.13)	0.27 (0.12)	0.078	0.152	**0.013**††
GR# ($\times10^3$/μL)	3.51 (0.86)	3.52 (1.03)	0.920	3.47 (0.87)	3.89 (1.04)	**0.006***	3.71 (1.06)	4.02 (1.27)	**0.046****	3.32 (1.04)	3.50 (1.16)	0.236	0.822	0.259
ERI ($\times10^3$/μL)	4.63 (0.30)	4.53 (0.33)	**0.032****	4.59 (0.40)	4.46 (0.32)	**0.001****	4.64 (0.27)	4.53 (0.26)	**0.003***	4.66 (0.42)	4.62 (0.55)	0.706	0.852	0.611
Hb (g/dL)	13.15 (0.80)	13.23 (0.71)	0.591	13.12 (1.07)	13.27 (0.90)	0.153	13.09 (0.77)	13.38 (1.08)	**0.038****	13.22 (0.62)	13.45 (1.04)	0.285	0.793	0.852
Hct (%)	41.66 (2.19)	40.42 (2.21)	**0.006***	41.65 (3.03)	40.00 (2.54)	**0.000***	42.02 (2.50)	40.75 (2.56)	**0.001****	42.20 (2.08)	41.91 (2.72)	0.555	0.897	0.073
MCV (fL)	90.22 (4.24)	89.34 (4.07)	**0.041***	90.82 (3.81)	89.97 (3.86)	**0.003***	90.75 (3.79)	89.92 (3.84)	**0.006***	91.00 (5.51)	90.53 (5.03)	0.068	0.932	0.822
MCH (pg)	28.46 (1.44)	29.24 (1.60)	**0.001***	28.60 (1.33)	29.86 (1.47)	**0.000***	28.29 (1.43)	29.53 (1.72)	**0.000***	28.54 (1.77)	29.19 (2.10)	0.092	0.868	0.477
MCHC (g/dL)	31.54 (0.84)	32.73 (0.77)	**0.000****	31.50 (0.71)	33.20 (0.73)	**0.000****	31.15 (0.64)	32.83 (1.13)	**0.000****	31.37 (0.51)	32.09 (1.45)	0.079	0.155	**0.044**††
RDW (%)	12.86 (0.53)	12.73 (0.49)	0.109	12.88 (0.56)	12.71 (0.72)	0.158	13.23 (0.81)	12.89 (0.78)	**0.005***	12.85 (0.50)	12.69 (0.58)	0.202	0.078	0.681
PL ($\times10^3$/μL)	197.44 (53.91)	198.64 (63.90)	0.467	191.75 (47.50)	187.64 (46.18)	0.190	212.00 (39.28)	206.34 (42.46)	0.347	182.10 (38.28)	173.55 (38.57)	0.079	0.134	0.056
MPV (fL)	8.72 (1.03)	8.46 (0.83)	**0.017***	8.70 (0.91)	8.48 (0.84)	**0.002***	9.02 (0.91)	8.76 (0.75)	**0.000****	8.42 (0.81)	8.28 (0.75)	0.058	0.149	0.154
PLR ratio	98.91 (32.19)	95.05 (30.01)	0.677	102.93 (35.01)	96.33 (27.49)	0.092	102.25 (35.63)	101.18 (35.09)	0.721	93.66 (30.84)	90.39 (28.26)	0.332	0.875	0.749
GLR ratio	1.77 (0.62)	1.71 (0.61)	0.530	1.88 (0.70)	2.06 (0.82)	**0.046****	1.78 (0.74)	1.95 (0.78)	0.103	1.67 (0.61)	1.78 (0.64)	0.247	0.610	0.365
SII	351.02 (147.93)	347.65 (187.99)	0.696	350.13 (117.95)	373.89 (129.63)	0.265	383.44 (185.45)	409.24 (182.16)	0.430	309.53 (142.73)	311.83 (138.38)	0.823	0.600	0.178

*Result obtained through T-Student test.

**Result obtained through Wilcoxon test.

†Results obtained through ANOVA e Tukey test.

††Results obtained through Kruskal Wallis e Bonferroni test.

Bold are intended to highlight the variables where statistically significant differences were found.

SD, standard deviation; AerG, continuous aerobic group; IntG, aerobic interval group; ComG, combined group; CG, control group; LEU, Leukocytes; LI, lymphocytes percentage; MO, monocytes percentage; GR, granulocytes percentage; LI#, lymphocytes gross value; MO#, monocytes gross value; GR#, granulocytes gross value; ERI, erythrocytes; Hb, hemoglobulin concentration; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean globular hemoglobulin; MCHC, mean corpuscular hemoglobulin concentration; RDW, erythrocytes distribution width; PL, platelets; MPV, mean platelet volume; PLR ratio, platelets to lymphocytes ratio; GLR, granulocytes to lymphocytes ratio; SII, systemic immune-inflammation index.

-4.0%) and the ComG ($p = 0.001$; Δ = -3.0%); MCV in the AerG ($p = 0.041$; Δ = -1.0%), the IntG ($p = 0.003$; Δ = -0.9%) and the ComG ($p = 0.006$; Δ = -0.9%); MPV in the AerG ($p = 0.017$; Δ = -3.0%), the IntG ($p = 0.002$; Δ = -2.5%) and the ComG ($p < 0.001$; Δ = -2.9%); RDW in the ComG ($p = 0.005$; Δ = -2.6%). The results for the inflammatory markers analyzed by each type of aquatic

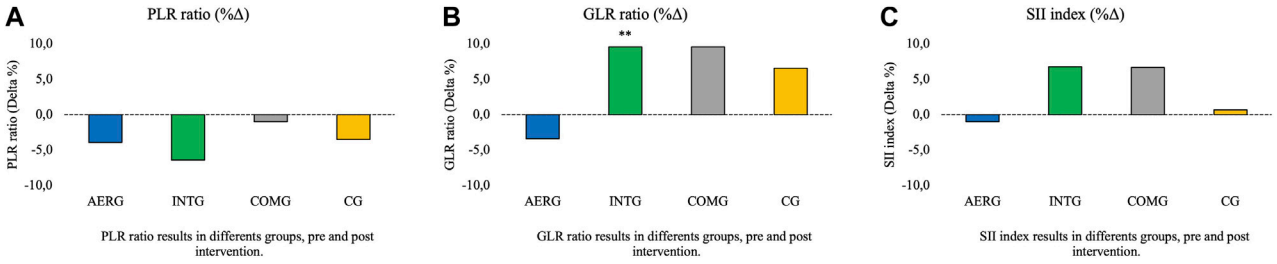

FIGURE 3
Percentage of delta (%Δ) the difference post-pre intervention for the immune inflammation markers PLR ratio, GLR ratio and SII index.

TABLE 3 Inflammatory markers levels at baseline and after 28 weeks of intervention.

	AerG			IntG			ComG			CG				
	M1	M2	Time (p)	M1	M2	Time (p)	M1	M2	Time (p)	M1	M2	Time (p)	Group (M1)	Group (M2)
	Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)		Mean (SD)	Mean (SD)			
IL-10 (pg/ml)	18.45 (14.08)	17.67 (15.30)	0.514	28.55 (10.13)	36.70 (6.85)	**0.000****	28.15 (14.12)	24.69 (10.04)	0.127	20.32 (12.87)	16.87 (9.10)	0.054	**0.005**††	**0.000**††
IL-1ra (pg/ml)	76.93 (51.79)	81.28 (58.52)	0.757	89.30 (56.68)	92.98 (69.54)	0.585	104.69 (63.82)	115.71 (67.19)	0.157	121.77 (83.56)	88.31 (59.96)	**0.008****	0.179	0.220
IL-1ß (pg/ml)	30.05 (3.75)	29.50 (3.31)	0.443	35.04 (16.67)	33.25 (17.15)	0.117	27.98 (10.16)	27.00 (14.27)	0.064	25.39 (4.04)	26.49 (4.24)	**0.043***	**0.010**††	**0.007**††
TNF-α (pg/ml)	37.38 (21.07)	28.65 (21.72)	**0.011****	17.65 (11.13)	14.27 (9.63)	**0.001****	69.01 (28.82)	52.89 (25.25)	**0.005***	31.98 (16.75)	32.59 (18.96)	0.936	**0.000**††	**0.000**††
TNF-α/ IL-10 ratio	5.00 (6.25)	3.85 (5.66)	0.056	0.89 (1.07)	0.45 (0.52)	**0.000****	2.55 (0.75)	2.23 (0.93)	0.125	3.18 (4.16)	3.18 (3.13)	0.351	**0.000**††	**0.000**††
IL-1β/IL-1ra ratio	0.63 (0.45)	0.75 (0.79)	0.657	0.67 (0.60)	1.01 (1.98)	0.982	0.42 (0.40)	0.36 (0.32)	**0.047****	0.35 (0.33)	0.50 (0.38)	0.053	**0.025**††	0.097

*Result obtained through T-Student test.
**Result obtained through Wilcoxon test.
†Results obtained through ANOVA e Tukey test.
††Results obtained through Kruskal Wallis and Bonferroni test.
Bold are intended to highlight the variables where statistically significant differences were found.
SD, standard deviation; AerG, continuous aerobic group; IntG, aerobic interval group; ComG, combined group; CG, control group; IL-10, Interleukin 10; IL-1ra, interleukin 1; IL-1ß, interleukin 1 beta; TNF-α, tumor necrosis factor; TNF-α/IL-10, ratio between tumor necrosis factor e Interleukin 10; IL-1β/IL-1ra, ratio between interleukin 1 beta e interleukin 1.

exercise program, before and after the intervention, are shown in Table 3.

Before the intervention (baseline) statistically significant differences were found between groups in the following inflammatory markers: IL-10 between the AerG and the ComG (p = 0.011), the AerG and the IntG (p = 0.002), and the IntG and the CG (p = 0.022)); IL-1ß between the CG and the AerG (p = 0.021), the CG and the IntG (p = 0.007), the ComG and the AerG (p = 0.045) and the ComG and the IntG (p = 0.015)); TNF-α between the IntG and the CG (p = 0.004), the IntG and the AerG (p < 0.001), the IntG and the ComG (p < 0.001), the CombG and the CG (p < 0.001), and the ComG and the AerG (p = 0.001)); TNF-α/IL-10 between the IntG and the CG (p = 0.001), the IntG and the AerG (p < 0.001), and the IntG and the ComG (p < 0.001); IL-1ß/IL-1ra between the CG and the IntG (p = 0.029), the CG and the AerG (p = 0.011), and the ComG and the AerG (p = 0.035)).

In the identified cases, the results were higher in the IntG and the ComG for IL-10, lower in the ComG and the CG for IL-1ß, lower in the IntG for TNF-α and the TNF-α/IL-10, and lower in the CG and the ComG for IL-1ß/IL-1ra.

After the intervention statistically significant differences were also found between groups in the following variables: IL-10 between the AerG and the ComG (p = 0.017), the AerG and the IntG (p < 0.001), the CG and the ComG (p = 0.042), the CG and the IntG (p < 0.001) and the ComG and the IntG (p < 0.001)); IL-1ß between the ComG and the IntG (p = 0.001), and the ComG and the AerG (p = 0.002)); TNF-α between the IntG and

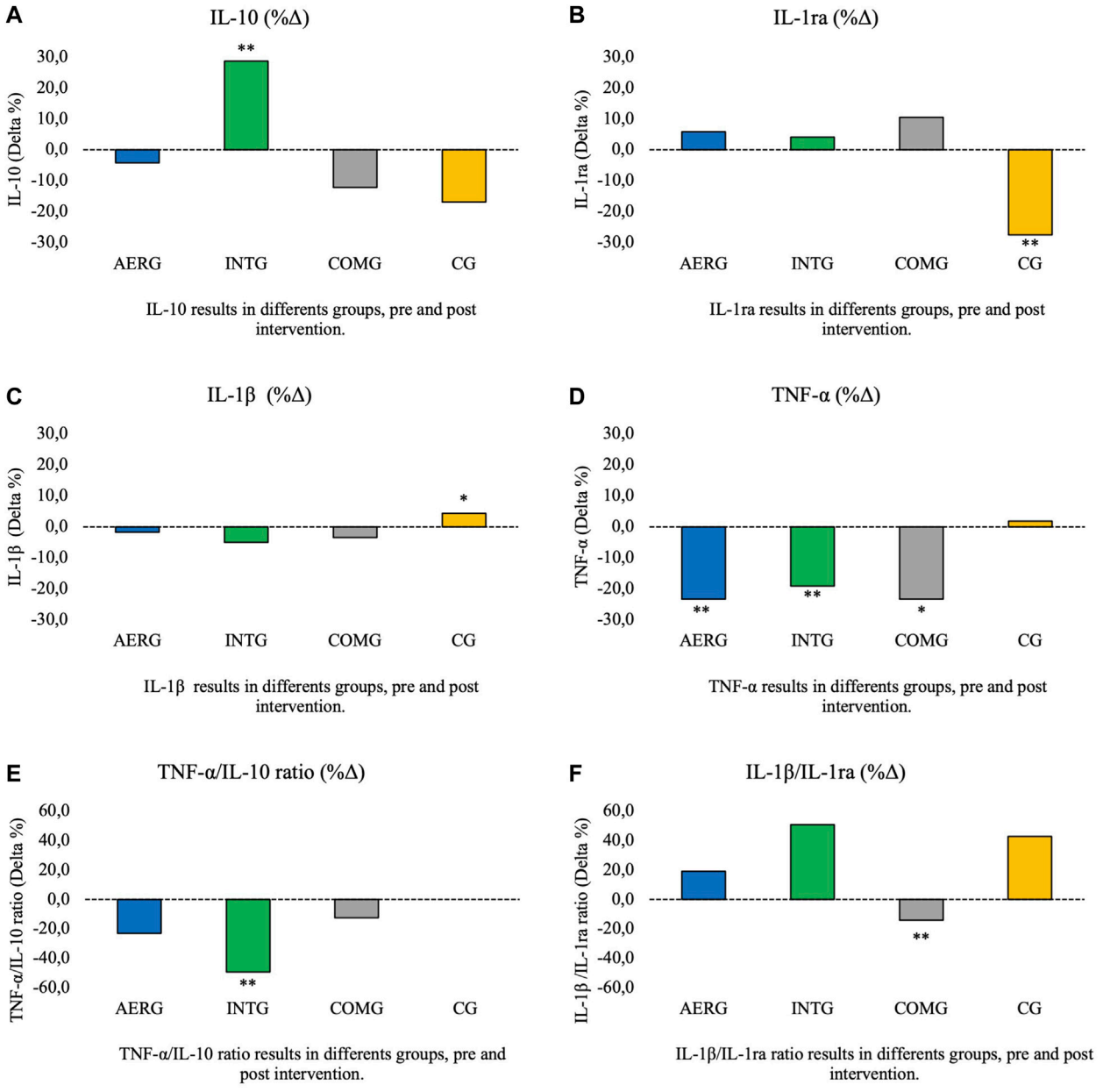

FIGURE 4
Percentage of difference post-pre intervention for cytokines IL-10, IL-1ra, IL-1β, TNF-α and inflammatory index TNFα-/IL-10, IL-1β/IL-1ra.

the AerG ($p = 0.001$), the IntG and the CG ($p < 0.001$), the ComG and the IntG ($p < 0.001$), the ComG and the AerG ($p = 0.001$), and the ComG and the CG ($p = 0.036$)); TNF-α/IL-10 between the IntG and the AerG ($p < 0.001$), the IntG and the ComG ($p < 0.001$), and the IntG and CG ($p < 0.001$). In the identified cases, the results were higher in the IntG for IL-10 and IL-1ß/IL-1ra, lower in the ComG for IL-1ß, and lower in the IntG for TNF-α.

The IL-10 levels showed a tendency to decrease in all groups, except for the IntG, which showed a significant statistical increase ($p < 0.01$; $\Delta = 28.5\%$). The IL-1ra levels showed a tendency to increase, in all exercise groups, but not in the CG, which showed a significant decrease ($p = 0.008$; $\Delta = -27.5\%$). For the IL-1ß levels, a tendency to decrease was found in all aquatic exercise groups, but not in the CG, where there was a significant increase ($p = 0.043$; $\Delta = 4.3\%$). The TNF-α levels showed a significant decrease in all exercise groups (AerG: $p = 0.011$; $\Delta = -23.4\%$; IntG $p = 0.001$; $\Delta = -19.1\%$; and ComG $p = 0.005$; $\Delta = -23.4\%$), but not in the CG. The results also showed a significant decrease in TNF-α/IL-10 ratio in the IntG ($p < 0.001$; $\Delta = -49.3\%$), and for the IL-1ß/IL-1ra ratio in the ComG ($p = 0.047$; $\Delta = -13.5\%$). (Figure 4).

4 Discussion

The purpose of the present study was to evaluate the impact of different aquatic exercise programs (continuous aerobic exercise, interval aerobic exercise and combined exercise) on the systemic hematologic and inflammatory markers of community dwelling elderly. A preliminary systematic bibliographic search revealed the innovative characteristics of the present study, since there were very few studies that had evaluated similar immune variables, in exercise performed in aquatic environment, in older persons.

Results for the systemic hematologic variables revealed significant statistical differences in most of the variables analyzed, due to the intervention with aquatic physical exercise (AerG, IntG and ComG), but no significant differences were found in the CG. A more detailed analysis of the results revealed significant differences over time (from M1 to M2), i.e., between pre- and post-intervention, in the AerG for ERI, Hct, MCV, MCH, MCHC and MPV, in the IntG for LEU, GR, ERI, Hct, MCV, MCH, MCHC and MPV, and in the ComG for LEU, GR, ERI, Hb, Hct, MCV, MCH, MCHC, RDW and MPV. The difference of the mean was higher for MCHC in the AerG (Δ = 3.8%) and for GR in the IntG (Δ = 12.1%) and in the ComG (Δ = 8.4%).

A study carried out by Chupel et al. (2017), that tested the effectiveness of a 28 weeks land-based muscle strength exercise program using elastic bands, in elderly women, showed that exercise intervention caused changes in hematologic variables, specifically significant increases in Hb, MCV, MCH and MCHC, and significant reductions in the LEU and LI variables. In the Hct and ERI variables, no significant statistical differences were found. Similar results had already been reported in Johannsen et al. (2012) study, using a 6-months intervention with aerobic exercise, that promoted significant reductions in the LEU and LI variables. Another recent study by Santos (2020) tested the effectiveness of an aquatic exercise program in elderly women for 1 year, and found a reduction in MCH, MCHC and PL, and an increase in Hct. However, those differences were not statistically significant. The Hb values remained unchanged.

The present study showed similar results to those reported by Chupel et al. (2017) in the three exercise groups (AerG, IntG and ComG), with significant increases in MCH and MCHC. The IntG and ComG groups showed the highest difference in average in both variables. Levels of Hb showed a tendency to increase in the three exercise groups, reaching statistically significance only in the ComG group.

In opposition to the results found in Chupel et al. (2017) study, present results revealed a significant reduction of MCV in the three exercise groups, with the highest variation of the mean being registered in the AerG, and an increase in the number of leucocytes in the IntG and ComG groups. The IntG registered the highest variation of the mean. Regarding the Hct and ERI variables, our results showed a statistically significant reduction in all aquatic exercise groups, with the IntG registering the highest difference of the mean in both cases, in opposition to the results obtained by Chupel et al. (2017). However, in Chupel's study the participants were much older and frail.

Present results clearly reveal a positive variation regarding hematologic health, with high levels of MCV and ERI being associated with a higher risk of death from all causes, especially cardiovascular and infectious diseases (Dratch et al., 2019). The increase in LEU numbers has positive implications at a hematologic level, since leucocytes have an important role in tissue recovery and host defense as well as antimicrobial properties (D'asta et al., 2018). Despite low levels of Hct being associated with the presence of anaemia, that can lead to the development of several chronic diseases (Suresh et al., 2021), the reduction of Hct values found in our study does not seem to have clinical implications since they remained within the clinical reference values (36–48% for women and 40–54% for men). Our results when compared to those of Santos (2020), found a significant statistical increase in MCH and MCHC, in all exercise groups, and in Hb in the ComG. Thus, present results showed a better effect on hematologic health.

Although some of the present results are somehow different from those found in the literature, the values remained within the normal limits, showing that aquatic exercise contributed to the maintenance of normal hematologic variables. The differences mentioned above may be directly related to the typology of physical exercise programs applied in different studies. In our study, the impact of different aquatic exercise programs (continuous aerobic exercise, interval aerobic exercise and combined exercise) was tested. Our research did not find any other intervention study that included these types of programs or variables, making it an important contribution to this field of research.

Although leucocyte numbers have been shown to increase with ageing (Hopkin et al., 2021), the total amount of LEU in the blood increases with acute physical exercise, i.e., exercise of short duration and high intensity (Gleeson et al., 2013). It is possible that our results may represent a cumulative effect of the acute bouts of exercise performed regularly for 28 weeks.

Regarding the inflammatory markers, significant differences were found for most of the analyzed variables, resulting from the aquatic physical exercise interventions. A more detailed analysis of the results revealed significant differences between the pre- and post-intervention phases for the: AerG, in TNF-α; for the IntG, in IL-10, TNF-α and TNF-α/IL-10; for the ComG, in TNF-α and IL-1β/IL-1ra. The differences of the means for the TNF-α variable was higher in the AerG and the ComG (Δ= -23.4% in both cases).

The results from the Chupel et al. (2017) study showed significant increases in IL-10 after a land-based muscle strength program. In another study published by the same authors (Chupel et al., 2018), a 14-weeks combined exercise program (aerobic exercise and muscle strength exercise) resulted in an increase in the anti-inflammatory markers IL-10 and IL-1ra. For the pro-inflammatory markers, results from the same study revealed a significant reduction in TNF-α and IL-1ß.

Another study by Furtado et al. (2020), showed a decrease in IL-1ß after 28-weeks of intervention with a combined exercise program. In aquatic environment, a study carried out by Korb et al. (2018), that tested the effectiveness of a 12-weeks aerobic walking program, showed a reduction in IL-10, while the IL-1ß levels were the same before and after the intervention. In a study by Ortega et al. (2012), that tested the effectiveness of an 8-months aquatic aerobic program, the results showed a decrease for the anti-inflammatory marker IL-10, and for the pro-inflammatory markers IL-1ß and for TNF-α. The results of another study by Da Silva et al. (2018) also showed a reduction in TNF-α after a 12-weeks intervention with a low-intensity aquatic exercise program. Still in an aquatic environment, in the study by Pochmann et al. (2018), the results showed significant increases for IL-1ra after 1 month of intervention with an aquatic physiotherapy program. Colato et al. (2017) applied a 12-week water walking program to a group of overweight women, and the results showed that, after the intervention, both IL-10 and TNF-α values increased significantly. In the same way, statistically significant increases were found for IL-10, after a 12-week aquatic walk/run intervention program (Korb et al., 2018), and TNF-α values also tended to increase after a water bike endurance program intervention (Bansi et al., 2013).

Overall, our results showed a tendency for the reduction in the pro-inflammatory markers IL-1β and TNF-α all the exercising groups. The plasma levels of the anti-inflammatory IL-10 increased in the IntG, while in the other groups the values showed a trend towards a decrease. Similar results were previously found in the studies by Colato et al. (2017) and Korb et al. (2018), after intervention with programs of walking/running in aquatic environment. Athletes do show increased levels of plasma IL-10 in response to high training volume and intensity (Minuzzi et al., 2017). The bouts of high intensity characteristic of the interval aerobic program may explain the differences between the exercising groups for this variable. It is possible that the small decrease of IL-10 found for the AerG and the ComG could also reflect a response to the decrease in TNF-α seen in these groups. As for the IL-1ra levels, our results showed an increasing trend in all exercise groups, whereas in the CG a significant statistical reduction was observed. Similar results had previously been found in the studies by Chupel et al. (2017) and Chupel et al. (2018), after an intervention with a land-based muscular strength and combined exercise program, and in the study by Pochmann et al. (2018), after an aquatic physiotherapy program intervention. Conversely, in the studies by Korb et al. (2018) and Ortega et al. (2012), the IL-10 levels were reduced with the intervention in an aquatic environment (walking exercise and aerobic exercise, respectively). For the IL-1ß variable, our results showed a trend towards a reduction in all exercise groups. However, there was a significant statistical increase in the CG. In the study by Korb et al. (2018), IL-1ß values remained unchanged after a 12-week intervention of walking/running in an aquatic environment. On the other end, for the TNF-α levels there was a significant statistical reduction in all exercise groups (AerG and ComG showed a higher means variation), while in the CG the TNF-α values showed a tendency to increase. Similar results were previously found by Chupel et al. (2018) and Furtado et al. (2020) after land exercise interventions and by Ortega et al. (2012) and Silva et al. (2018) after exercise interventions in aquatic environments. However, the results of Colato et al. (2017) and Bansi et al. (2013) showed increases in TNF-α values after intervention with water walking and water bike endurance, respectively.

The above findings are reflected in the TNF-α/IL-10 inflammatory index tendency to decrease in all aquatic exercise groups, reaching statistically significance in the IntG. For the IL-1β/IL-1ra ratio a statistically significant reduction in the ComG was also found. Our results confirm the results already found in the study by Chupel et al. (2018), where reductions in the TNF-α/IL-10 and IL-1β/IL-1ra ratios also occurred, after intervention with physical exercise on land. No studies were found to assess these ratios in an aquatic environment. Our results suggest that physical exercise in an aquatic environment induces an anti-inflammatory response, reducing chronic low-grade inflammation, and contributing to the reduction in the development of metabolic and cardiovascular diseases. In the study by Kumari et al. (2018), the TNF-α/IL-10 ratio was associated with the risk of coronary artery disease, suggesting that this ratio may play a vital role in the development of this type of pathologies.

Regarding the markers PLR, GLR and SII, few studies were found where the effect of exercise was reported. As for the PLR ratio, our results showed a downward trend in all exercising groups. No intervention studies were found in which the impact of long-term exercise on this marker was evaluated, however, the results found in the studies by Bessa et al. (2016) and Korkmaz et al. (2018), showed that PLR ratio values increased after evaluating the acute effect of physical exercise. Regarding the GLR ratio, our results showed a statistically significant increase for IntG, which is in line with the results found in the study by Svendsen et al. (2016), where high-intensity physical exercise provided significant increases in the NLR ratio. Additionally, our results showed a tendency towards a reduction in the GLR ratio in the AerG, corroborating the results found in the study by Makras et al. (2005), where a moderate-intensity exercise program caused a significant reduction in the NLR values. These data reinforces the idea that higher-intensity physical exercise provides greater increases in NLR ratio, compared to moderate-intensity exercise programs (Walzik et al., 2021). Finally, for SII in the present study there was a visible tendency to increase in the IntG and ComG and to decrease in the AerG. These results contradict those found in the study by Joisten et al. (2021), where SII values were reduced after an intervention with a high-intensity interval exercise program. However, studies that evaluated the acute effect of exercise on SII showed that values increased after higher intensity exercise (Walzik et al., 2021).

Our results showed that physical exercise caused a buffering effect, both in anti-inflammatory and in pro-inflammatory markers, since the results in the exercise groups followed a positive trend towards a less inflammatory environment, while the opposite occurred in the CG where the low-grade chronic inflammation increased. These results provide evidence that aquatic physical exercise programs will improve/mantain the inflammatory profile.

The results of the present study suggest that the three aquatic exercise programs can effectively improve immune variables in community dwelling elderly participants. Some of the changes observed in our study are similar to those found in studies carried out on land environments. Thus, exercise in aquatic environments can be seen as a viable alternative to land-based exercise, especially when other health constraints are installed (e.g. orthopedic, rheumatological or functional limitations, circulatory disease, vertigo, etc.). As for the type of exercise program, the AerG had a higher variation of the mean for TNF-α, the IntG had a higher variation of the mean for IL-10 and IL-1ß, and the ComG had a higher variation of the mean for IL-1ra and TNF-α (the same value as AerG).

5 Limitations

Regarding the limitations of the study, a block randomization methodology could have been used, which would have avoided such an unequal number of participants between groups. According to the purpose of the study, the magnitude of the variation should have been tested statistically, giving more robustness to the results. In the statistical procedure we could have applied a repeated-measures ANOVA. However, due to lack of normality and highly skewed data, nonparametric procedure guarantees higher statistical power.

6 Conclusion

The results of the present study allow us to conclude that the participation in physical exercise aquatic environment programs can lead to beneficial changes in the systemic hematologic variables of community dwelling elderly.

Regarding the inflammatory markers, the present results showed that, in general, all exercise groups decreased their pro-inflammatory markers levels as well as their inflammatory index TNF-α/IL-10, while the opposite effect was found in the CG. This means that the participation of community dwelling elderly in aquatic physical exercise programs caused a buffering effect, contributing not only to the maintenance, but also to an improvement in their inflammatory profile.

As for the type of aquatic program that best benefited the systemic hematological and inflammatory markers, the results were not totally conclusive, since different exercise programs led to improvements in different variables. All showed important benefits in decreasing chronic low-grade inflammation. However, the combined aquatic exercise program showed significant statistical improvements in a higher number of systemic hematologic variables and a decrease in TNF-α levels, while the interval aerobic aquatic exercise program showed significant statistical improvements in a greater number of inflammatory markers, including an increase in IL-10 and a decrease in TNF-α levels.

Further studies assessing systemic hematologic and inflammatory markers among older participants in physical exercise interventions in aquatic environments are needed, aiming to better clarify the effects of exercise on chronic low-grade inflammation.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee for Health of the Faculty of Sport Sciences and Physical Education, University of Coimbra (reference: CE/FCDEF-UC/00462019). The patients/participants provided their written informed consent to participate in this study.

Author contributions

CF, HS, FMS, and MC-R participated in data collection and data organization. CF and BO analyzed the data. CF wrote the manuscript. JPF, AMT, JS, and FMS reviewed the manuscript. JPF, AMT, and JS coordinated the research. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the FCT—CIDAF, UID/PTD/04213/2019.

Acknowledgments

The authors would like to thank all the persons that accepted to participate in the intervention study and to the Municipality of Serta, namely to Paulo Luis for the relevant contribution to this project, providing access to the participants and permission for

the data collection in the different water-based exercise community programs.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Abd El-Kader, S., and Al-Shreef, F. (2018). Inflammatory cytokines and immune system modulation by aerobic versus resisted exercise training for elderly. *Afr. Health Sci.* 18 (1), 120–131. doi:10.4314/ahs.v18i1.16

ACSM (2018). *Diretrizes do ACSM para os testes de esforço e sua prescrição - 10a Edição*.

Bansi, J., Bloch, W., Gamper, U., Riedel, S., and Kesselring, J. (2013). Endurance training in MS: Short-term immune responses and their relation to cardiorespiratory fitness, health-related quality of life, and fatigue. *J. Neurol.* 260 (12), 2993–3001. doi:10.1007/s00415-013-7091-z

Baptista, F., and Sardinha, L. (2005). *Avaliação da aptidão física e do equilíbrio de pessoas idosas. Baterias de Fullerton*. Cruz Quebrada, Portugal: Faculdade de Motricidade Humana.

Bessa, A., Oliveira, V., Agostini, G., Oliveira, R., Oliveira, A., White, G., et al. (2016). Exercise intensity and recovery: Biomarkers of injury, inflammation, and oxidative stress. *J. Strength Cond. Res.* 30 (2), 311–319. doi:10.1519/JSC.0b013e31828f1ee9

Chupel, M., Direito, F., Furtado, G., Minuzzi, L., Pedrosa, F., Colado, J., et al. (2017). Strength training decreases inflammation and increases cognition and physical fitness in older women with cognitive impairment. *Front. Physiol.* 8, 377. doi:10.3389/fphys.2017.00377

Chupel, M., Minuzzi, L., Furtado, G., Santos, M., Hogervorst, E., Filaire, E., et al. (2018). Exercise and taurine in inflammation, cognition, and peripheral markers of blood-brain barrier integrity in older women. *Appl. Physiology, Nutr. Metabolism = Physiologie Appliquee, Nutr. Metabolisme* 43 (7), 733–741. doi:10.1139/apnm-2017-0775

Colato, A., Fraga, L., Dorneles, G., Vianna, P., Chies, J., and Peres, A. (2017). Impact of aerobic water running training on peripheral immune-endocrine markers of overweight-obese women. *Sci. Sports* 32 (1), 46–53. doi:10.1016/j.scispo.2016.04.003

Da Silva, L., Menguer, L., Motta, J., Dieke, B., Mariano, S., Tasca, G., et al. (2018). Effect of aquatic exercise on mental health, functional autonomy, and oxidative dysfunction in hypertensive adults. *Clin. Exp. Hypertens.* 40 (6), 547–553. doi:10.1080/10641963.2017.1407331

D'asta, F., Halstead, F., Harrison, P., Orlandini, S., Moiemen, N., and Lord, J. (2018). The contribution of leucocytes to the antimicrobial activity of platelet-rich plasma preparations: A systematic review. *Platelets* 29 (1), 9–20. doi:10.1080/09537104.2017.1317731

Dratch, A., Kleine, C., Streja, E., Soohoo, M., Park, C., Hsiung, J., et al. (2019). Mean corpuscular volume and mortality in incident hemodialysis patients. *Nephron* 141 (3), 188–200. doi:10.1159/000495726

Dziubek, W., Bulińska, K., Rogowski, Ł., Gołębiowski, T., Kusztal, M., Grochola, M., et al. (2015). The effects of aquatic exercises on physical fitness and muscle function in dialysis patients. *Biomed. Res. Int.* 2015, 912980. doi:10.1155/2015/912980

Ferreira, J., Teixeira, A., Serrano, J., Farinha, C., Santos, H., Silva, F., et al. (2020). Impact of aquatic-based physical exercise programs on risk markers of cardiometabolic diseases in older people: A study protocol for randomized-controlled trials. *Int. J. Environ. Res. Public Health* 17 (22), 8678. doi:10.3390/ijerph17228678

Franceschi, C., Garagnani, P., Parini, P., Giuliani, C., and Santoro, A. (2018). Inflammaging: A new immune-metabolic viewpoint for age-related diseases. *Nat. Rev. Endocrinol.* 14 (10), 576–590. doi:10.1038/s41574-018-0059-4

Franklin, B., Whaley, M., Howley, E., and Balady, G. (2000). *ACSM's guidelines for exercise testing and prescription*. Lippincott Williams & Wilkins.

Franz, F., Erdfelder, E., and Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* 7 (10), 175–191. doi:10.3758/BF03193146

Furtado, G., Chupel, M., Minuzzi, L., Rama, L., Colado, J., Hogervorst, E., et al. (2020). The mediating effect of different exercise programs on the immune profile of frail older women with cognitive impairment. *Curr. Pharm. Des.* 26, 906–915. doi:10.2174/1381612826666200203123258

Gleeson, M., Bishop, N., and Walsh, N. (2013). "Exercise immunology," in *Routledge 2 park square* (Milton Park: Abingdon). Oxon OX14 4RN (Routledge).

Hopkin, S., Lord, J., and Chimen, M. (2021). Dysregulation of leukocyte trafficking in ageing: Causal factors and possible corrective therapies. *Pharmacol. Res.* 163, 105323. doi:10.1016/j.phrs.2020.105323

Johannsen, N., Swift, D., Johnson, W., Dixit, V., Earnest, C., Blair, S., et al. (2012). Effect of different doses of Aerobic exercise on total white blood cell (WBC) and WBC subfraction number in postmenopausal women: Results from DREW. *PLoS ONE* 7 (2), e31319–9. doi:10.1371/journal.pone.0031319

Joisten, N., Proschinger, S., Rademacher, A., Schenk, A., Bloch, W., Warnke, C., et al. (2021). High-intensity interval training reduces neutrophil-to-lymphocyte ratio in persons with multiple sclerosis during inpatient rehabilitation. *Mult. Scler.* 27 (7), 1136–1139. doi:10.1177/1352458520951382

Kapasi, Z., Ouslander, J., Schnelle, J., Kutner, M., and Fahey, J. (2003). Effects of an exercise intervention on immunologic parameters in frail elderly nursing home residents. *J. Gerontol. A Biol. Sci. Med. Sci.* 58 (7), 636–643. doi:10.1093/gerona/58.7.m636

Karvonen, M., Kentala, E., and Mustala, O. (1957). The effects of training on heart rate; a longitudinal study. *Ann. Med. Exp. Biol. Fenn.* 35 (3), 307–315.

Korb, A., Bertoldi, K., Lovatel, G., Delevatti, R., Elsner, V., Meireles, L., et al. (2018). Acute exercise and periodized training in different environments affect histone deacetylase activity and interleukin-10 levels in peripheral blood of patients with type 2 diabetes. *Diabetes Res. Clin. Pract.* 141, 132–139. doi:10.1016/j.diabres.2018.04.037

Korkmaz, A., Yıldız, A., Duyuler, P., Duyuler, S., Yılmaz, S., Basyigit, F., et al. (2018). Combination of change in hematological parameters with exercise stress test to predict coronary artery disease. *J. Clin. Lab. Anal.* 32 (1), e22205. doi:10.1002/jcla.22205

Krüger, K. (2017). Inflammation during obesity–pathophysiological concepts and effects of physical activity. *Dtsch. Z. Sportmed.* 68, 163–169. doi:10.5960/dzsm.2017.285

Kumari, R., Kumar, S., Ahmad, M., Singh, R., Pradhan, A., Chandra, S., et al. (2018). TNF-α/IL-10 ratio: An independent predictor for coronary artery disease in North Indian population. *Diabetes Metab. Syndr.* 12 (3), 221–225. doi:10.1016/j.dsx.2017.09.006

Makras, P., Koukoulis, G., Bourikas, G., Papatheodorou, G., Bedevis, K., Menounos, P., et al. (2005). Effect of 4 weeks of basic military training on peripheral blood leucocytes and urinary excretion of catecholamines and cortisol. *J. Sports Sci.* 23 (8), 825–834. doi:10.1080/02640410400021815

Minuzzi, L., Rama, L., Bishop, N., Rosado, F., Martinho, A., Paiva, A., et al. (2017). Lifelong training improves anti-inflammatory environment and maintains the number of regulatory T cells in masters athletes. *Eur. J. Appl. Physiol.* 117 (6), 1131–1140. doi:10.1007/s00421-017-3600-6

Moreira, N., da Silva, L., and Rodacki, A. (2020). Aquatic exercise improves functional capacity, perceptual aspects, and quality of life in older adults with musculoskeletal disorders and risk of falling: A randomized controlled trial. *Exp. Gerontol.* 142, 111135. doi:10.1016/j.exger.2020.111135

Nisansala, T., Weerasekera, M., Ranasinghe, N., Marasinghe, C., Gamage, C., Fernando, N., et al. (2021). Contributing role of TNF, IL-10, sTNFR1 and TNF gene polymorphisms in disease severity of leptospirosis. *Med. Microbiol. Immunol.* 210 (4), 211–219. doi:10.1007/s00430-021-00714-5

Ortega, E., Bote, M. E., Giraldo, E., and Garcia, J. J. (2012). Aquatic exercise improves the monocyte pro- and anti-inflammatory cytokine production balance in fibromyalgia patients. *Scand. J. Med. Sci. Sports* 22 (1), 104–112. doi:10.1111/j.1600-0838.2010.01132.x

Pedersen, B., and Fischer, C. (2007). Beneficial health effects of exercise--the role of IL-6 as a myokine. *Trends Pharmacol. Sci.* 28 (4), 152–156. doi:10.1016/j.tips.2007.02.002

Pochmann, D., Peccin, P., da Silva, I., Dorneles, G., Peres, A., Nique, S., et al. (2018). Cytokine modulation in response to acute and chronic aquatic therapy intervention in Parkinson disease individuals: A pilot study. *Neurosci. Lett.* 674, 30–35. doi:10.1016/j.neulet.2018.03.021

Rikli, R., and Jones, J. (1999). Development and validation of a functional fitness test for community-residing older adults. *J. Aging Phys. Act.* 7, 129–161. doi:10.1123/japa.7.2.129

Rodriguez, I., Ruiz, N., León, M., Enríquez, L., Velásquez, M., Aguirre, J., et al. (2020). Immunosenescence study of T cells: A systematic review. *Front. Immunol.* 11, 604591. doi:10.3389/fimmu.2020.604591

Santos, H. (2020). *Avaliação de parâmetros vasculares e hemodinâmicos numa população idosa - efeito do exercício continuado*. 978-989-8252-48-7.

Sellami, M., Gasmi, M., Denham, J., Hayes, L., Stratton, D., Padulo, J., et al. (2018). Effects of acute and chronic exercise on immunological parameters in the elderly aged: Can physical activity counteract the effects of aging? *Front. Immunol.* 9, 2187. doi:10.3389/fimmu.2018.02187

Shimizu, K., Suzuki, N., Imai, T., Aizawa, K., Nanba, H., Hanaoka, Y., et al. (2011). Monocyte and T-cell responses to exercise training in elderly subjects. *J. Strength Cond. Res.* 25 (9), 2565–2572. doi:10.1519/JSC.0b013e3181fc5e67

Simpson, R., Kunz, H., Agha, N., and Graff, R. (2015). Exercise and the regulation of immune functions. *Prog. Mol. Biol. Transl. Sci.* 135, 355–380. doi:10.1016/bs.pmbts.2015.08.001

Souza, D., Vale, A., Silva, A., Araújo, M., Júnior, C., Lira, C., et al. (2021). Acute and chronic effects of interval training on the immune system: A systematic review with meta-analysis. *Biology* 10 (9), 868. doi:10.3390/biology10090868

Spielmann, G., McFarlin, B., O'Connor, D., Smith, P., Pircher, H., and Simpson, R. (2011). Aerobic fitness is associated with lower proportions of senescent blood T-cells in man. *Brain Behav. Immun.* 25 (8), 1521–1529. doi:10.1016/j.bbi.2011.07.226

Suresh, K., Wang, K., Sethi, I., Zhang, B., Margalit, A., Puvanesarajah, V., et al. (2021). Spine surgery and preoperative hemoglobin, hematocrit, and hemoglobin A1c: A systematic review. *Glob. Spine J.* 2192568220979821, 155–165. doi:10.1177/2192568220979821

Svendsen, I., Killer, S., Carter, J., Randell, R., Jeukendrup, A., and Gleeson, M. (2016). Impact of intensified training and carbohydrate supplementation on immunity and markers of overreaching in highly trained cyclists. *Eur. J. Appl. Physiol.* 116 (5), 867–877. doi:10.1007/s00421-016-3340-z

Walzik, D., Joisten, N., Zacher, J., and Zimmer, P. (2021). Transferring clinically established immune inflammation markers into exercise physiology: Focus on neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and systemic immune-inflammation index. *Eur. J. Appl. Physiol.* 121 (7), 1803–1814. doi:10.1007/s00421-021-04668-7

Weltevrede, M., Eilers, R., Melker, H., and Baarle, D. (2016). Cytomegalovirus persistence and T-cell immunosenescence in people aged fifty and older: A systematic review. *Exp. Gerontol.* 77, 87–95. doi:10.1016/j.exger.2016.02.005

Werner, C., Hecksteden, A., Morsch, A., Zundler, J., Wegmann, M., Kratzsch, J., et al. (2019). Differential effects of endurance, interval, and resistance training on telomerase activity and telomere length in a randomized, controlled study. *Eur. Heart J.* 40 (1), 34–46. doi:10.1093/eurheartj/ehy585

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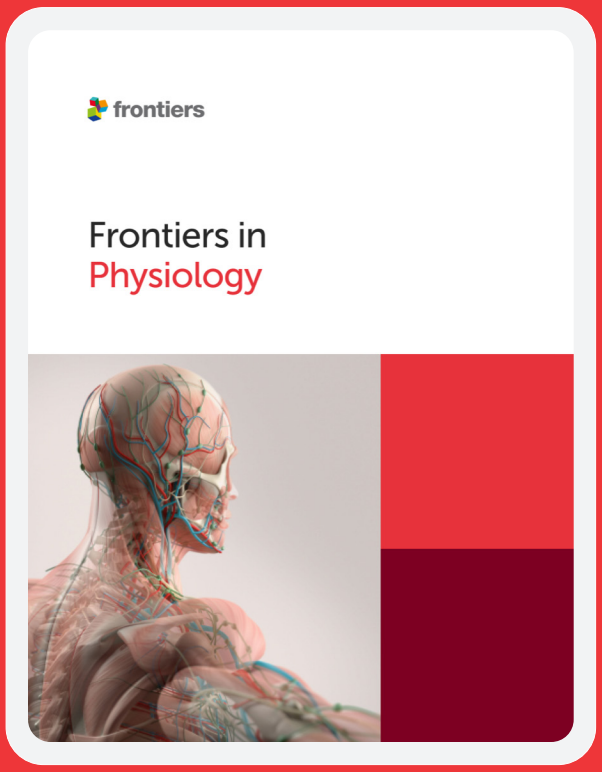

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