frontiers | Research Topics

Metabolic responses and adaptations to exercise

Edited by
Simone Luti, Pantelis Theodoros Nikolaidis, Tania Gamberi and Cristina Vassalle

Coordinated by
Alessio Pellegrino

Published in
Frontiers in Physiology
Frontiers in Sports and Active Living

ISSN 1664-8714
ISBN 978-2-8325-4912-4
DOI 10.3389/978-2-8325-4912-4

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Metabolic responses and adaptations to exercise

Topic editors
Simone Luti — University of Florence, Italy
Pantelis Theodoros Nikolaidis — University of West Attica, Greece
Tania Gamberi — University of Florence, Italy
Cristina Vassalle — Gabriele Monasterio Tuscany Foundation (CNR), Italy

Topic coordinator
Alessio Pellegrino — University of Florence, Italy

Citation
Luti, S., Nikolaidis, P. T., Gamberi, T., Vassalle, C., Pellegrino, A., eds. (2024). *Metabolic responses and adaptations to exercise*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-4912-4

Table of contents

frontiers | Frontiers in Sports and Active Living

TYPE Editorial
PUBLISHED 08 May 2024
DOI 10.3389/fspor.2024.1416649

OPEN ACCESS

EDITED AND REVIEWED BY
Giuseppe D'Antona,
University of Pavia, Italy

*CORRESPONDENCE
Simone Luti
✉ simone.luti@unifi.it

RECEIVED 12 April 2024
ACCEPTED 29 April 2024
PUBLISHED 08 May 2024

CITATION
Gamberi T, Vassalle C, Nikolaidis PT, Pellegrino A and Luti S (2024) Editorial: Metabolic responses and adaptations to exercise.
Front. Sports Act. Living 6:1416649.
doi: 10.3389/fspor.2024.1416649

Editorial: Metabolic responses and adaptations to exercise

Tania Gamberi[1], Cristina Vassalle[2], Pantelis Theodoros Nikolaidis[3], Alessio Pellegrino[4] and Simone Luti[1]*

[1]Department of Experimental and Clinical Biomedical Sciences, School of Mathematical, Physical and Natural Sciences, University of Florence, Florence, Italy, [2]Department of Laboratory Medicine, Gabriele Monasterio Tuscany Foundation (CNR), Pisa, Italy, [3]School of Health and Welfare Sciences, University of West Attica, Athens, Greece, [4]Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy

KEYWORDS

acute exercise, chronic exercise, sport metabolism, healthy aging, performance

Editorial on the Research Topic
Metabolic responses and adaptations to exercise

Sedentary lifestyle is one of the main problems of modern society and is responsible for the dramatic growth of overweight and obesity, which are also powerful risk factors for serious chronic diseases. Conversely, physical exercise is proposed as a highly effective tool of treating and preventing the main causes of morbidity and mortality—associated with aging—in industrialized countries. Numerous epidemiological and prospective studies have reported a strong association between physical activity and morbidity–mortality index of population, even in overweight and obese person.

In fact, several metabolic changes occur in the organism during exercise, leading to the activation of adaptive mechanisms. These mechanisms aim to establish a new dynamic equilibrium especially at the metabolic level, which enhances health and optimize performance in elite athletes.

Clearly, many variables (e.g., exercise mode, frequency, duration, and intensity) may affect the results.

What molecular mechanisms are activated in response to physical exercise? And in recovery, do they act similarly? How regular physical exercise is associate with health, especially in elderly people? What benefits arise from physical activity as an expression and activation of molecular mechanisms?

The present Research Topic aimed to address the above-mentioned research questions including ten scientific papers.

Chmielecki et al. examined the effect of an exhaustive run on Interleukin IL-4, IL-8, IL-10, and Tumor Necrosis Factor α concentrations in 16 amateur athletes, and observed that, although IL-4 did not change after exercise, its baseline value negatively correlated with post-exercise luminescence. Based on these findings, the researchers concluded that plasma IL-4 might be related to preservation of an optimal balance between oxidants and antioxidants during and after exercise.

Abdalla-Silva et al. investigated the role of the β2-Adrenoceptors (β2-ARs) blockade on the acute molecular responses induced by a single session of resistance exercise in rodent skeletal muscles. Considering their findings, the researchers suggested that β2-ARs stimulation during acute resistance exercise stimulated the hypertrophic gene Nr4a3 and inhibited the overexpression of atrophic genes in the first hours of

postexercise recovery, highlighting the impact of the sympathetic nervous system on muscle adaptations.

In another study, Meihua et al. studied sweat samples using chemical isotope labeling liquid chromatography-mass spectrometry before and after high-intensity interval exercise-induced fatigue in 14 long-distance runners and identified 446 metabolites and the sweat metabolome group. They concluded that alterations of hypoxanthine concentration in sweat might be used as a biomarker for the diagnosis of exercise-induced fatigue, whereas the change of pyruvate concentration in sweat might be used as a discriminant index for the energy metabolism mode of the body pre- and post-exercise.

Ye et al. studied the impact of acute exercise on the metabolome and transcriptome profiles in mice skeletal muscle, by using an integrative and holistic approach combining multi-omics technologies. Results revealed the complex network between metabolites and genes, identifying 34 differentially expressed metabolites (28 up-regulated and 6 down-regulated), as well as 245 differentially expressed genes (155 up-regulated and 90 down-regulated genes), involved in different metabolic and signaling pathways related to the exercise-induced physiological regulation of skeletal muscle. These data help to better understand the molecular changes that follow exercise in skeletal muscle using omics technologies, evidencing innovative potential future therapeutic targets related with exercise.

Jia et al. discussed the role of exerkines in osteoarthritis, some with beneficial other with adverse effects in the disease (e.g., irisin, lactate, secreted frizzled-related protein, neuregulin, and adiponectin favourable, while IL-6, IL-7, IL-15, IL-33, myostatin, fractalkine, follistatin-like 1, visfatin, activin A, migration inhibitory factor, apelin and growth differentiation factor-15 with aggravating effects). These exerkines can be modulated by exercise with effects on the disease (although some type or load in terms of intensity and frequency of exercise can elicit adverse exerkines as well) and can serve as new promising pharmacological targets to develop future personalized interventional strategies.

Wang et al. explored the effects and underlying mechanisms of exercise in treating lumbar disc herniation (characterised by lumbar disc degeneration, annulus fibrosus rupture, herniation of the nucleus pulposus irritate and nerve compression, resulting in lumbar and/or lower extremity pain). Three main mechanisms by which exercise appears to exert its benefits were discussed: mechanical compression, inflammatory chemical stimulation, and autoimmunity, whereas the use of non-acute self-weighted exercise types, exercise durations exceeding two weeks, and non-high-intensity exercise therapies appear, in view of actual available evidence, more effective to reduce disease severity and alleviate symptoms.

Meloni et al. measured fat oxidation rates and cardiorespiratory responses during exercise in trained and recreational athletes with post-acute sequelae of SARS-CoV2-infection; results, although requiring further confirmation in additive future studies, suggested that specific endurance training, when compared with a simply active lifestyle, might be more protective against alterations caused by virus infection, such as mitochondrial dysfunction or, more in general, abnormalities of the fatty acid oxidation pathway.

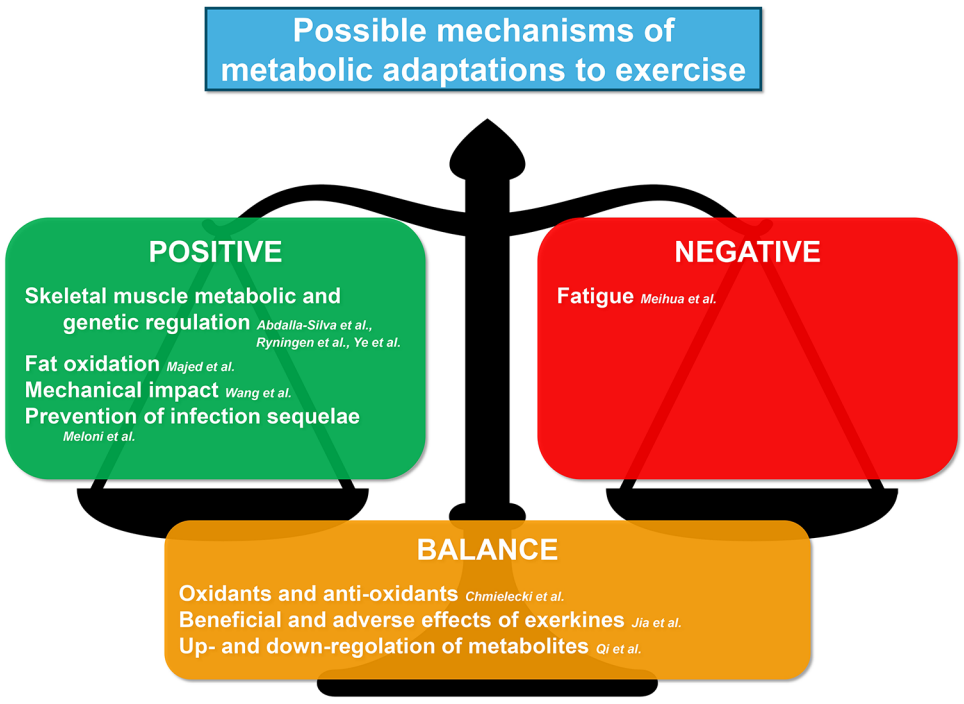


FIGURE 1
Summary of the contribution of this Research Topic to understanding the possible mechanisms of metabolic adaptations to exercise.

Qi et al. summarized the progress in the application of metabolomics in sport science. They discussed the advantage and disadvantage of the different chemical analysis platform and data analysis. In this review the authors pointed out the role of metabolomics in searching for potential biomarkers and therapeutic targets by detecting metabolite changes in a variety of biological fluids and tissues.

Majed et al. studied the metabolic, perceptual, spatiotemporal and gait stability parameters of walking around the preferred speed (PWS) in thirty-four healthy and sedentary volunteers (18 women and 16 men) aged between 18 and 26 years. Main findings indicated the increase of fat oxidation suggesting benefits of walking at the preferred walking speed for sedentary young adults compared to walking at a slower or faster intensity.

Ryningen et al. analyzed the expression levels of circulating microRNAs in elite cyclists after heavy strength (HS) and short-sprint (SS) training methods with the aim to find potential biomarkers for individual optimal restitution time. They found that both training methods can increase the circulating levels of some of the miRNAs associated with muscle development such as myomiRs miR-1-3p, 133a-3p and 133b-3p suggesting their relevance as measures of acute response and recovery status.

Collectively, the published manuscripts in this Research Topic have contributed meaningful novel findings as summarized in Figure 1, but there are still many unsolved issues in metabolic responses and adaptations to exercise and more research on physical activity effects are needed. The final goal is to advance our mechanistic understanding of exercise metabolism in tissues and systems throughout the body, explore the therapeutic benefits of exercise, and integrate these findings with other emerging themes across the metabolic arena. This knowledge may help to better target interventions preserving health and fitness and managing diseases, as well as to improving performance in athletes.

Author contributions

TG: Writing – review & editing. CV: Writing – review & editing. PN: Writing – review & editing. AP: Writing – review & editing. SL: Writing – review & editing, Writing – original draft.

Acknowledgments

We would like to express our sincere gratitude to all the authors and reviewers who contributed to this Research Topic.

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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 06 October 2023
DOI 10.3389/fphys.2023.1273342

OPEN ACCESS

EDITED BY
Simone Luti,
University of Florence, Italy

REVIEWED BY
Elen H. Miyabara,
University of São Paulo, Brazil
Maurice H. T. Ling,
Temasek Polytechnic, Singapore

*CORRESPONDENCE
Renyi Liu,
renyi.liu@cug.edu.cn

RECEIVED 06 August 2023
ACCEPTED 25 September 2023
PUBLISHED 06 October 2023

CITATION
Ye X, Liu R, Qiao Z, Chai X and Wang Y (2023), Integrative profiling of metabolome and transcriptome of skeletal muscle after acute exercise intervention in mice.
Front. Physiol. 14:1273342.
doi: 10.3389/fphys.2023.1273342

Integrative profiling of metabolome and transcriptome of skeletal muscle after acute exercise intervention in mice

Xing Ye[1], Renyi Liu[1]*, Zhixian Qiao[2], Xiaocui Chai[2] and Yan Wang[2]

[1]School of Physical Education, China University of Geosciences (Wuhan), Wuhan, China, [2]Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China

This study aims to explore the molecular regulatory mechanisms of acute exercise in the skeletal muscle of mice. Male C57BL/6 mice were randomly assigned to the control group, and the exercise group, which were sacrificed immediately after an acute bout of exercise. The study was conducted to investigate the metabolic and transcriptional profiling in the quadriceps muscles of mice. The results demonstrated the identification of 34 differentially expressed metabolites (DEMs), with 28 upregulated and 6 downregulated, between the two groups. Metabolic pathway analysis revealed that these DEMs were primarily enriched in several, including the citrate cycle, propanoate metabolism, and lysine degradation pathways. In addition, the results showed a total of 245 differentially expressed genes (DEGs), with 155 genes upregulated and 90 genes downregulated. KEGG analysis indicated that these DEGs were mainly enriched in various pathways such as ubiquitin mediated proteolysis and FoxO signaling pathway. Furthermore, the analysis revealed significant enrichment of DEMs and DEGs in signaling pathways such as protein digestion and absorption, ferroptosis signaling pathway. In summary, the identified multiple metabolic pathways and signaling pathways were involved in the exercise-induced physiological regulation of skeletal muscle, such as the TCA cycle, oxidative phosphorylation, protein digestion and absorption, the FoxO signaling pathway, ubiquitin mediated proteolysis, ferroptosis signaling pathway, and the upregulation of KLF-15, FoxO1, MAFbx, and MuRF1 expression could play a critical role in enhancing skeletal muscle proteolysis.

KEYWORDS

acute exercise, metabolome, transcriptome, skeletal muscle, quadriceps, mice

1 Introduction

Skeletal muscle, being the most abundant tissue in the body, is indispensable for movement as the body relies on the contraction and relaxation of skeletal muscle. Furthermore, a key predictor of mortality is muscle mass quality (Baskin et al., 2015). The skeletal muscle plays a crucial role in maintaining the balance of metabolic processes, making it one of the primary sites for glucose disposal (DeFronzo and Tripathy, 2009). Unhealthy lifestyles and habits have led to an alarming increase in the incidence of various chronic diseases, imposing a significant economic burden on healthcare systems worldwide (Kim et al., 2016; Farr and Khosla, 2019; Castellanos et al., 2020). It is well known that exercise, as a stressor, has a significant impact on physical function (Cook et al., 2012; Wang et al., 2021). Moreover, exercise is a major factor in the extensive metabolic and

molecular remodeling of skeletal muscles (Egan and Zierath, 2013). The skeletal muscle undergoes various adaptations in response to exercise, including the synchronization of muscle contraction and ATP production, as well as the utilization of energy triggered by mechano- and other metabolic sensors (Egan and Zierath, 2013; Camera et al., 2016; Fan and Evans, 2017).

Studies have shown that regular physical activity enhances exercise performance, induces increased skeletal muscle protein synthesis, inhibits skeletal muscle protein degradation (Qiu et al., 2014), improves mitochondrial function, and subsequently promotes metabolic homeostasis in the body (Shen et al., 2021). However, intensive or prolonged exercise can trigger an oxidative imbalance, which can cause oxidative damage and lead to muscle fatigue (Reid et al., 1992; O'Neill et al., 1996). If the ability to remodel skeletal muscles decreases and their quality deteriorates, it can affect the body's motor function and increase the risk of falls and injuries. The enhancement of muscle function is achieved through improved the exercise capacity and the optimized metabolic functions. Despite significant efforts to uncover the comprehensive changes in muscle caused by exercise, there are still many regulators of skeletal muscle that have yet to be discovered.

Transcriptomic profiling is a powerful technique that allows for the identification of intricate gene expression patterns (Wang et al., 2009). On the other hand, metabolomics serves as a valuable tool in detecting and elucidating changes in metabolites within a biological system under varying conditions (Dettmer et al., 2007), but few studies have investigated the comprehensive transcriptomic and metabolomic profile of exercise muscular physiology. The objective of this study was to evaluate and analyze the transcriptional and metabolic networks of skeletal muscle that are regulated by exercise in mice. By doing so, we were able to gain insights into the mechanisms through which exercise intervention impacts the overall metabolic balance of skeletal muscle.

2 Material and methods

2.1 Animals

The study used C57BL/6 male mice were 9 weeks old and weighing 22.1 ± 1.3 g. The mice utilized in this study were housed in the animal facilities located at Hubei Provincial Center for Disease Prevention and Control in China, where they received a standard photoperiod of 12-h light/12-h dark cycles. Mice were housed under standard conditions, with access to food and water unrestricted, at a temperature of 21°C ± 1°C. After 1 week of the acclimation period, the animals were randomly assigned to the control or exercise groups. All animal experiments were approved by the local Animal Care and Use Committee and performed following the recognized guidelines for laboratory animal care and management. Ethical approval was granted by the Academic Integrity and Scientific Research Ethics Committee of China University of Geosciences (Wuhan).

2.2 Aerobic capacity test and treadmill exercise protocol

This study utilized a mouse treadmill, following the method previously mentioned by Kruger et al. (2009) and Kemi et al. (2002). To minimize the stress caused by the equipment, the animals were provided with 1 week of exercise adaptation prior to undergoing the exercise test. Each animal underwent an incremental exercise test to exhaustion at least 4 days prior to commencing the experiments to measure their maximal oxygen uptake ($\dot{V}O_2max$) and maximal running speed (Vmax). The mice were acclimated in the treadmill chamber for 10 min before undergoing a continuous, progressive exercise test until exhaustion. The test involved a 5-min warm-up at a speed of 0.20 m/s, followed by an increase in running speed by 0.05 m/s every 3 min until the mice reached a state of exhaustion. The animals were randomly divided into two groups, each consisting of six animals. The mice from the control group were exposed to the noise produced by the treadmill without engaging in actual running. On the other hand, the animals in the exercise group underwent a single running test until exhaustion at an intensity of 80% VO_2max, which corresponded to a speed of 0.40 ± 0.30 m/s.

2.3 Tissue samples

After conducting the exercise tests, the animals were administered anesthesia, and then euthanized using cervical dislocation. The muscle samples used in this study referenced the experimental conditions described previously (Song et al., 2023). The posterior thigh of mice was sterilized, and a skin incision was made. The m. gluteus superficialis and the tensor fasciae latae muscle were identified. A sample was taken from the exposed quadriceps femoris muscle. The quadriceps were weighed; mass was normalized to body mass. To maintain its integrity, the specimen was promptly cooled in a pre-chilled diethylpyrocarbonate (DEPC) solution at a temperature of 4°C, effectively eliminating any superficial blood and hair. Then, the muscular tissue was desiccated using filter paper, carefully placed into a cryopreservation tube, and promptly placed in liquid nitrogen for subsequent examination.

2.4 Experimental methods of metabolomics

Retrieve the sample from liquid nitrogen and place it on ice to thaw until it reaches a state where it can be easily cut (all subsequent steps were performed on ice). Chop and mix the samples, and weigh the samples at 20 mg (±1 mg) into the corresponding numbered centrifuge tubes. Add a steel ball using forceps, homogenize for 20 s with a ball mill (30 HZ), and centrifuge the sample at 3,000 r/min for 30 s at 4°C (the time can be increased according to the actual homogenization). After centrifugation, add 400 μL of 70% methanolic water internal standard extract, centrifuged at 2,500 r/min for 5 min, and let stand on ice for 15 min. Centrifuge at 12,000 r/min for 10 min at 4°C, transfer 300 μL of the supernatant to another centrifuge tube with a corresponding number, and let it stand in a −20°C refrigerator for 30 min; Under 4°C conditions, centrifuge again at 12,000 r/min for 3 min, transfer 200 μL of the supernatant into a lined tube inside the corresponding sample bottle for analysis on the machine. The samples were then analysed using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). Mass spectroscopy data processing was performed using Analyst software (version 1.6.3). Orthogonal projections to latent structures-

TABLE 1 Primer information for RT-qPCR.

Gene	Primer sequence (5′ to 3′)	
	F	R
MAFbx	CAGCTTCGTGAGCGACCTC	GGCAGTCGAGAAGTCCAGTC
KLF-15	GAGACCTTCTCGTCACCGAAA	GCTGGAGACATCGCTGTCAT
UCP3	CTGCACCGCCAGATGAGTTT	ATCATGGCTTGAAATCGGACC
Atf3	GAGGATTTTGCTAACCTGACACC	TTGACGGTAACTGACTCCAGC
Sap30	CAACTCTGTTGTTTGCGGGAG	AGATGCCTTGCACTCTTATCCA
β-actin	GTGACGTTGACATCCGTAAAGA	GCCGGACTCATCGTACTCC

discriminant analysis (OPLS-DA) were employed to analyze the differentially expressed metabolites (DEMs). DEMs were screened based on a combination of fold change, *t*-test *p*-value, and variable importance in the projection (VIP) values from the OPLS-DA model. The screening criteria were set as VIP >1 and *p*-value <0.05. Use R language to perform OPLS-DA to determine the magnitude of differences between samples. Subsequently, metabolic pathway enrichment analysis of DEMs was performed.

2.5 RNA-seq experimental method

Total RNA was isolated from skeletal muscle using the RNeasy Mini Kit (Qiagen, Hilden, Germany), and the quantity and purity were determined with a UV-Vis spectrophotometer (ND-1000, Nano-Drop Technologies, Rockland, United States). During RNA separation, the RNase-free DNase set (Qiagen, Hilden, Germany) was used to perform on-column DNase digestion, ensuring the elimination of any potential DNA contamination. The transcriptomic sequencing (RNA-seq) was performed using Illumina Novaseq 6000 (Illumina, CA) to construct and sequence libraries. The high-quality data were aligned to a transcript reference obtained from the mouse reference genome (version mm10), through Bowtie2 default parameters (Langmead and Salzberg, 2012). Transcript abundance was normalized and quantified utilizing RSEM (RNA-Seq by Expectation Maximization) (Li and Dewey, 2011). Differentially expressed genes (DEGs) were detected using a threshold of Fold Change ≥2 and FDR <0.01. Subsequently, the obtained DEGs were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional annotation and enrichment analysis.

2.6 Validation of DEGs by quantitative real-time PCR

To validate the reliability of RNA-seq data, a subset of 5 differentially expressed genes (DEGs) was selected for quantitative real-time PCR (qPCR) using the primers provided in Table 1. The High-capacity cDNA reverse transcription kit (Applied Biosystems) was used according to the manufacturer's instructions to synthesize cDNA from the RNA samples. The cDNA was obtained using the T100 PCR thermal cycler (Bio-Rad Laboratories, Munich, Germany) and used for qPCR. The iQ SYBR Green Supermix (Bio-Rad Laboratories, Munich, Germany) was utilized for qPCR to measure the mRNA expression. The amplification products were quantified using an iCycler (Bio-Rad Laboratories, Munich, Germany). The reaction conditions included an initial denaturation at 95°C for 3 min, followed by 42 cycles of denaturation at 95°C for 15 s, annealing at 61°C for 30 s, and elongation at 72°C for 30 s. The mRNA expression levels were normalized to the expression of the housekeeping gene *β*-actin. The results were analyzed using the $2^{-\Delta(\Delta Ct)}$ method and presented as fold changes relative to the control group.

2.7 Combined analysis of transcriptome and metabolome

Conjoint Analysis of DEGs and DEMs is conducted based on the relative content data from transcriptomics and metabolomics. Enrichment analysis is performed on the significantly enriched KEGG pathways for both DEGs and DEMs. This analysis identifies the pathways that are jointly enriched by DEGs and DEMs, and these pathways are plotted as bar graphs.

2.8 Statistical analysis

This study presents the mean values and standard errors of the mean, which were determined. The data was tested for normality using the Shapiro-Wilk test, and the results indicated that the data followed a normal distribution. A one-way ANOVA test was conducted to analyze the differences between the groups, and the results were considered statistically significant at $p < 0.05$. All statistical analyses were performed using SPSS 26.0 software.

3 Results

3.1 Screening for differential metabolites

The OPLS-DA model showed that the two groups of samples could be separated, indicating that the intervention of an acute bout

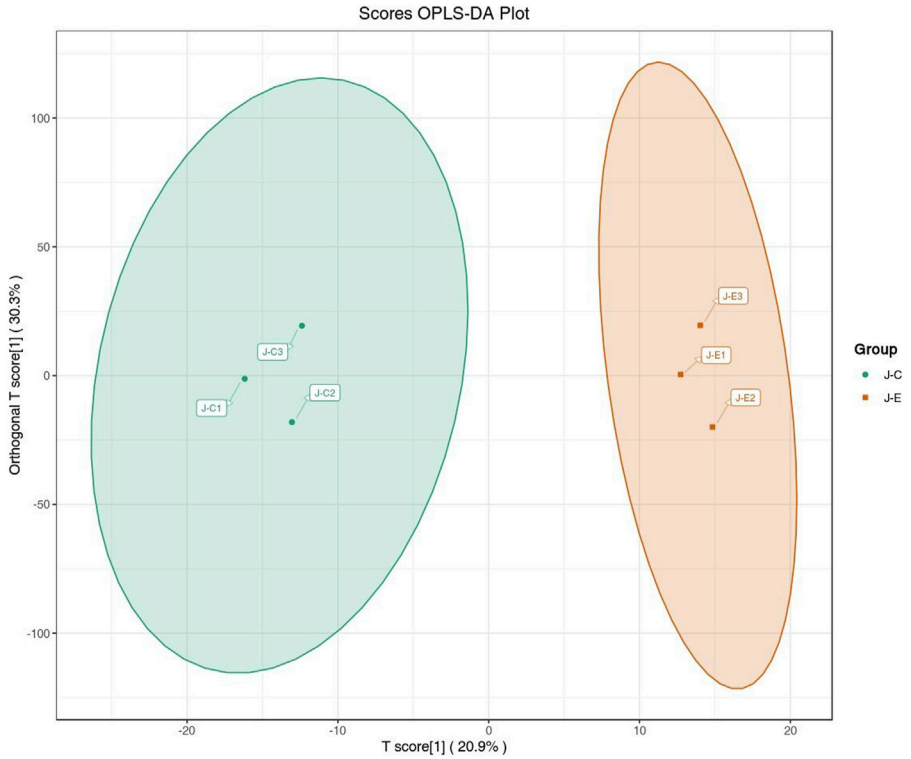


FIGURE 1
Score plot of the OPLS-DA. The abscissa indicates the predicted principal component; the ordinate indicates the orthogonal principal component; the percentage indicates the explanation rate of this component to the data set. Each point in the graph indicates a sample, and samples of the same group are represented using the same color.

of exercise caused changes in skeletal muscle metabolism in rats (Figure 1). The T score was 20.9% and the orthogonal T score was 30.3% indicating significant differences in metabolites between the experimental group and the control group. The fold change was combined with the VIP values of the OPLS-DA model to filter the DEMs. In the exercise group and the control group, a total of 34 DEMs were identified, comprising 28 upregulated metabolites and 6 downregulated metabolites (Figure 2). These include 15 amino acids and its metabolites, 8 organic acids and its derivatives, 3 nucleotide and its metabolites, 3 fatty acyls, 2 carbohydrates and its metabolites, 1 heterocyclic compound, 1 coenzyme and vitamin, and 1 aldehyde, ketone, and ester.

3.2 Signaling pathway analysis of differential metabolites

Metabolic pathway analysis was conducted on the DEMs between the exercise group and the control group, and a significant enrichment pathway for metabolites was obtained using a hypergeometric distribution algorithm ($p < 0.05$) (Figure 3). The top 20 pathways in terms of p-value were selected for display from smallest to largest and made into a bubble chart. The DEMs were mainly enriched in propanoate metabolism, protein digestion and absorption, d-amino acid metabolism, lysine degradation, citrate cycle, alanine, aspartate and glutamate metabolism, ferroptosis, pyruvate metabolism and aminoacyl-tRNA biosynthesis.

3.3 Screening of differential gene expression

The raw data were obtained from 6 sequenced samples of skeletal muscle total RNA samples after sequencing by the transcriptome Illumina Novaseq 6000 sequencing platform. Using Fold Change≥2 and FDR<0.01 as criteria, we identified 245 DEGs in the exercise group compared with the control group. Among them, 155 upregulated genes and 90 downregulated genes accounted for 63% and 37% of the total DEGs, respectively (Figure 4). Furthermore, we have identified 10 key genes that are highly associated with skeletal muscle (Table 2).

3.4 GO and KEGG analysis of differentially expressed genes

Based on our screened DEGs, the top 15 GO Terms in q-value ranking from GO functional analysis were selected for presentation and bubble plotted (Figure 5). The DEGs were annotated to skeletal muscle cell differentiation, protein folding chaperone, aging, response to muscle stretch, positive regulation of cell migration and response to heat in the biological process functional classification. The 245 differential genes were subjected to KEGG enrichment analysis, and the results showed that a total of 238 signaling pathways were enriched. The top 15 enriched KEGG signaling pathways were selected for display (Figure 6). These include the ubiquitin mediated proteolysis, longevity

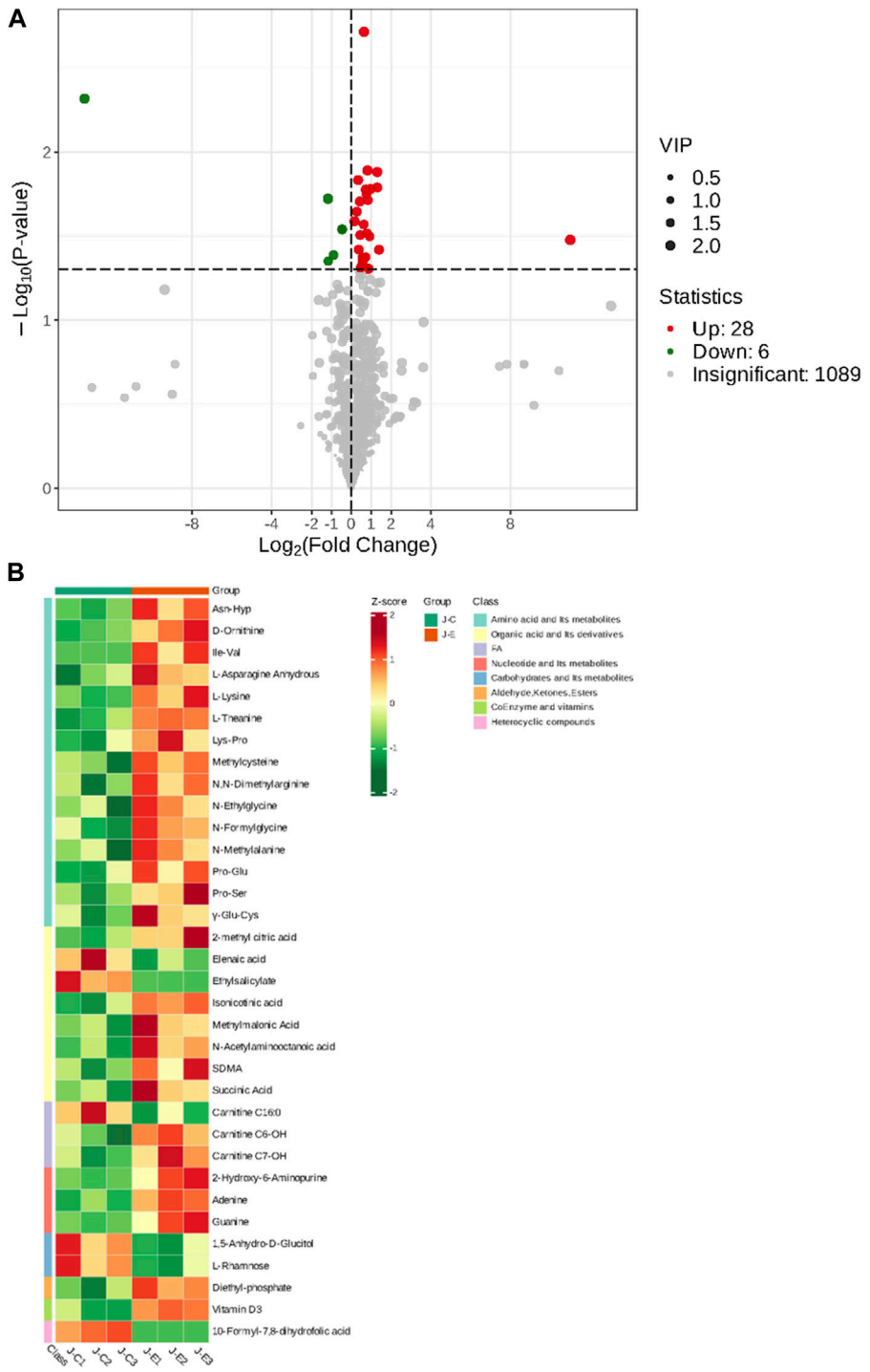


FIGURE 2
Screening of Differentially Expressed Metabolites. **(A)**: Volcano plot of differentially expressed metabolites. Each point in the volcano plot represents a metabolite, with green dots representing downregulated differential metabolites, red dots representing upregulated differential metabolites, and gray representing metabolites that are detected but not significantly different. The abscissa represents the logarithmic value (log2FC) of the relative abundance difference of a metabolite between the two groups of samples. The larger the absolute value of the abscissa, the greater the relative abundance difference of the metabolite between the two groups of samples. Under the filtering conditions of VIP + FC + *p*-value, the ordinate represents the significance level of the difference (-log10*p*-value), and the size of the dots represents the VIP value. **(B)**: Clustering heat map of the metabolites. The abscissa is used to display the names of samples, and the ordinate on the right is used to display the names of metabolites. Different colors are filled with different values obtained after normalization for different relative contents. Red indicates high content; green indicates low content.

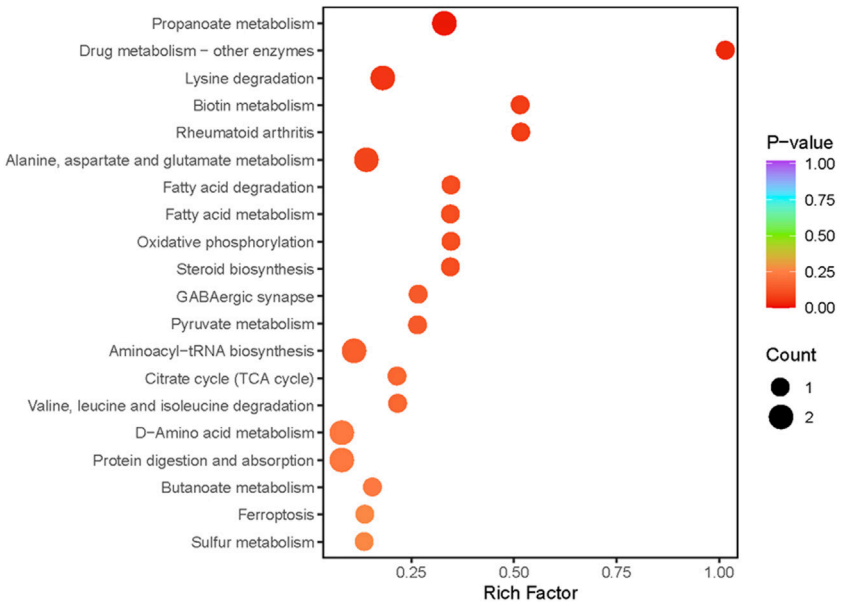


FIGURE 3
Pathway analysis of differentially expressed metabolites. The abscissa indicates the Rich Factor for each pathway, the ordinate is the pathway name (sorted by *p*-value), and the color of the dot reflects the *p*-value size, the redder the enrichment is, the more significant the enrichment is. Dot size relates to the number of differentially expressed metabolites.

regulating pathway, cAMP signaling pathway, FoxO signaling pathway, and MAPK signaling pathway.

3.5 Integrative KEGG pathway analysis of the transcriptome and metabolome

To further understand the correlation between metabolites and genes involved in the same biological process, we conducted a comprehensive analysis of transcriptomic and metabolomic data, resulting in the identification of 20 KEGG signaling pathways (Figure 7). The KEGG pathways predominantly enriched in differentially expressed metabolites and genes include protein digestion and absorption, biosynthesis of amino acids, cAMP signaling pathway, protein digestion and absorption and ferroptosis, among others.

3.6 Quantitative real-time PCR

The results of qPCR analysis in the samples are graphically represented in Figure 8. The levels of mRNA in the exercise mice were analyzed and compared to those of control animals. The transcript levels of MAFbx, Atf3, and Sap30 ($p < 0.01$ vs. CG, n = 3), KLF-15 and UCP3 ($p < 0.05$ vs. CG, n = 3) were significantly increased in the exercise group. The results of RNA-Seq are consistent with these findings.

4 Discussion

Skeletal muscle, a crucial component in controlling movement, plays a fundamental role as the primary regulator of metabolism in various body systems. Its susceptibility to the metabolic regulations imposed by exercise is particularly notable. To the best of our knowledge, this study represents the first investigation that employs transcriptomics and metabolomics to extensively explore the collective effects of acute exercise on skeletal muscle. This study identified a total of 34 metabolites that exhibited differential regulation. These metabolites are primarily associated with several metabolic pathways, including the citrate cycle (TCA cycle), oxidative phosphorylation, and Valine, leucine and isoleucine degradation. This transcriptomics analysis unveiled 245 differentially expressed genes. The KEGG pathways involved in this study include FoxO, ubiquitin mediated proteolysis signaling pathway. By conducting a joint analysis of transcriptomics and metabolomics, 20 enriched KEGG pathways were identified, including biosynthesis of amino acids, ferroptosis, protein digestion and absorption signaling pathway. Based on the aforementioned findings, we focus on a comprehensive analysis to investigate the potential mechanism by which exercise regulates skeletal muscle function.

To begin with, the results of metabolomics in this study demonstrated a significant increase in succinate levels within the TCA cycle following acute exercise, as illustrated in Figure 3. Succinate, being an integral component of the TCA cycle, can accumulate when the flow of succinic acid within the cycle is hindered. The metabolic dysfunction in the TCA cycle can lead to a deterioration in physiological function. Succinate is generated within the mitochondria and its main destiny upon transition to an aerobic environment is to undergo oxidation at the same location. Consequently, in a scenario where aerobic metabolism significantly outweighs anaerobic metabolism, succinate will not accumulate. However, the accumulation of succinate under aerobic conditions can occur if oxygen cost far exceeds the individual's maximal oxygen uptake (VO_2max) and anaerobic energy production is significant

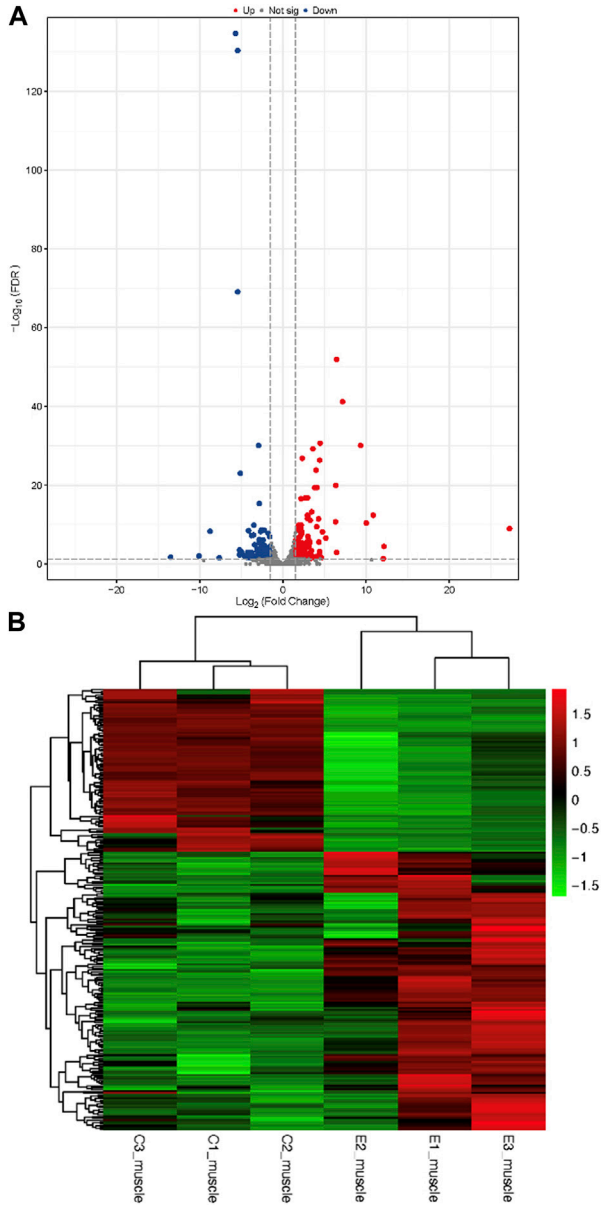


FIGURE 4
Identification of differentially expressed genes **(A)**: Volcano plots of differentially expressed genes. In a volcano plot, each dot represents a gene. Blue dots represent the downregulated genes, red dots represent the upregulated genes, and the gray dots represent the non-significant genes **(B)**: Heatmap of differentially expressed genes.

(Hochachka and Dressendorfer, 1976). In the analysis of metabolic pathways, succinate is not only closely related to the TCA cycle, but is also involved in various other metabolic pathways, including oxidative phosphorylation (Zhao et al., 2017).

The TCA cycle is an important pathway for intracellular oxidative phosphorylation to produce ATP, and mitochondria are the main site where the TCA cycle takes place. Moderate-intensity exercise can improve mitochondrial function, whereas both high-intensity and exhaustive exercise may disrupt mitochondrial structure and cause mitochondrial disorders (Tondera et al., 2009; Schoepe et al., 2012). In particular, skeletal muscle is an important site for the uptake and utilization of fatty acids, and mitochondria play an important role in cellular energy metabolism, being the main site for energy conversion, the TCA cycle, and oxidative phosphorylation. The mitochondria undergo pathological changes following acute exercise, leading to impaired function (Magalhaes et al., 2013). As a result, the cellular capacity for oxidative phosphorylation is affected. This implies that the disruption of mitochondria by acute exercise may lead to an inadequate energy supply and adversely affect the organism.

Disruptions in mitochondrial function could be related to amino acid pool imbalances and altered amino acid metabolism (Naseer et al., 2020). The fascinating finding in this study was that strenuous exercise resulted in the degradation of amino acids such as valine, leucine, isoleucine, and so on (as depicted in Figure 3), while amino acids such as glycine and serine increased, demonstrating that the body undergoes degradation and production of proteins associated with the injury zone immediately after the onset of the injury. It is believed that succinate is produced as a result of the catabolism of amino acids. The findings of this study demonstrated that the levels of 15 amino acids and their derivatives, such as l-asparagine anhydrous, l-lysine, d-ornithine, pyroglutamic acid, and n, n-dimethylarginine and so on, were upregulated following an acute bout of intensive exercise. These changes in metabolites involve pathways such as d-amino acid metabolism, pyruvate metabolism, propanoate metabolism, and fatty acid degradation and so on. Besides, a source of anaerobic energy may be generated by amino acid catabolism (Hochachka and Dressendorfer, 1976). The noteworthy elevation of amino acids *in vivo* following acute exercise suggests that the rate of acid production in the skeletal muscle of exercising mice exceeds the rate of elimination, there is an increase in muscle protein degradation, which results in the release of amino acids from muscle tissue into the bloodstream. This phenomenon could be a compensatory mechanism to provide amino acids as a substrate for energy production and gluconeogenesis during exercise. Exercise could stimulate the activity of key enzymes involved in amino acid catabolism (Harris et al., 2005).

The general consensus is that protein synthesis in skeletal muscle is suppressed during exercise, as evidenced by findings in both humans and other species (Rennie et al., 1980; Dohm et al., 1985). The results of transcriptomics in this study show that acute exercise could induce an increase in KLF-15, FoxO1, MAFbx, and MuRF1 expression (Table 2). KLF-15, a key transcription factor, not only inhibits skeletal muscle protein synthesis but also plays a crucial role in enhancing skeletal muscle proteolysis by promoting MuRF1 and MAFbx transcription, and stimulates FoxO1 (Kuo et al., 2013). FoxO1, in particular, is the main regulatory factor among all FoxO subtypes, expressed significantly in skeletal muscle. This study demonstrates that the activation of the FoxO signaling pathway is significantly enhanced, as evidenced by the data presented in Figure 6. In the process of KLF-15 mediated skeletal muscle degradation, FoxO plays a pivotal role as a transcription factor and serves as a central component of multiple degradation pathways. Participating in moderate or vigorous physical activity leads to an intensified generation of reactive oxygen species (ROS) (Vezzoli et al., 2016). The FoxO signaling pathway may be activated as a result of increased generation of ROS (Dodd et al., 2010). The importance of this heightened FoxO signaling lies in its role in increasing FoxO nuclear translocation, therefore leading to an

TABLE 2 Results of analysis of differential expression of key candidate genes.

Gene	Description	FDR	Up/down
MuRF1	Muscle RING-finger protein-1	1.82287E-09	up
MAFbx	Muscle Atrophy F-box protein	5.30082E-05	up
KLF-15	Kruppel-like factor 15	0.00078	up
FoxO1	Forkhead box O1	1.69647E-05	up
Tigar	Trp53 induced glycolysis regulatory phosphatase	2.71345E-09	down
Srl	Sarcalumenin	1.9239E-135	down
Sirt6	Sirtuin 6	0.00903	up
UCP3	Uncoupling protein 3 (mitochondrial, proton carrier)	1.09267E-08	up
Atf3	Activating transcription factor 3	1.10252E-52	up
HSP70	Heat shock protein70	6.8673E-42	up

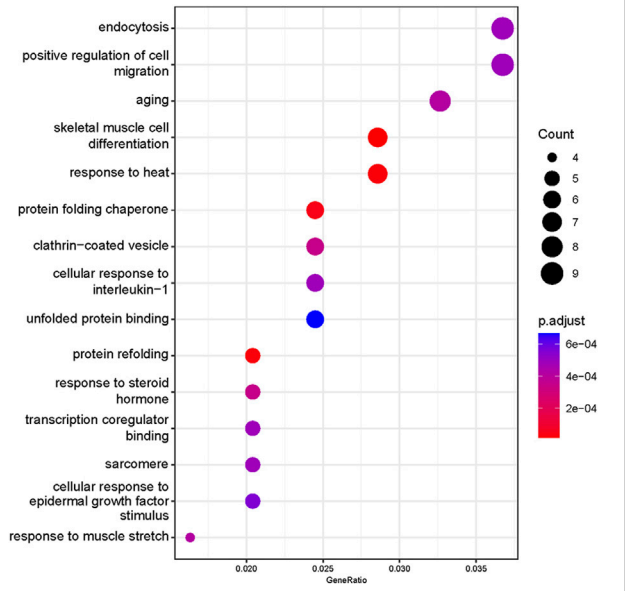


FIGURE 5
Gene Ontology enrichment analysis of differentially expressed genes. The abscissa is the ratio of the number of genes to all genes for the term, ordinate shows the GO terms, the size of the bubble represents the number of genes enriched in the GO terms, and color showed the q-value of GO terms.

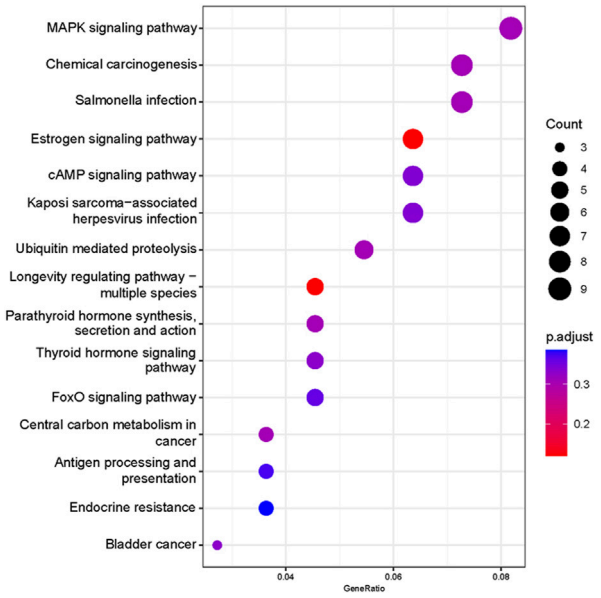


FIGURE 6
Functional annotation of the KEGG pathway for differentially expressed genes. Ordinate: nomenclature of the KEGG signaling pathway; Abscissa: gene numbers that annotated this pathway and the ratio of KEGG in all genes that annotated relative to the total KEGG.

intensified transcription of FoxO target genes, such as MAFbx and MuRF-1, which are directly involved in muscle catabolism (Bodine et al., 2001; Gomes et al., 2001; Crow, 2002), and MAFbx and MuRF-1 play a crucial role in specifically targeting proteins for degradation by the proteasome (Bodine et al., 2001; Gomes et al., 2001; Puthanveetil et al., 2013). In addition, we found that the HSP70, UCP3 and SIRT6 genes were significantly upregulated in skeletal muscle after exercise in this study. The increase in expression of the differential gene HSP70 can induce a protective anti-apoptotic response, alleviate membrane protein denaturation caused by injury stimuli, and protect the protein synthesis pathway in cells from damage (Mosser et al., 2000; Mokranjac et al., 2006). This high expression of UCP3 can reduce the release of ROS and prevent damage to the body (Nabben et al., 2008; Toime and Brand, 2010), while SIRT6 overexpression inhibits the protein degradation pathway related to muscle atrophy (Samant et al., 2017). However, their protective effects may not be sufficient to counteract the physical damage caused by acute exercise.

The ubiquitin-proteasome system (UPS) is the primary pathway for intracellular protein degradation (Meng et al., 2014). The importance of the ubiquitination level of proteins for quality control is further highlighted by the fact that approximately 80% of proteins in the body are degraded by the ubiquitin-proteasome

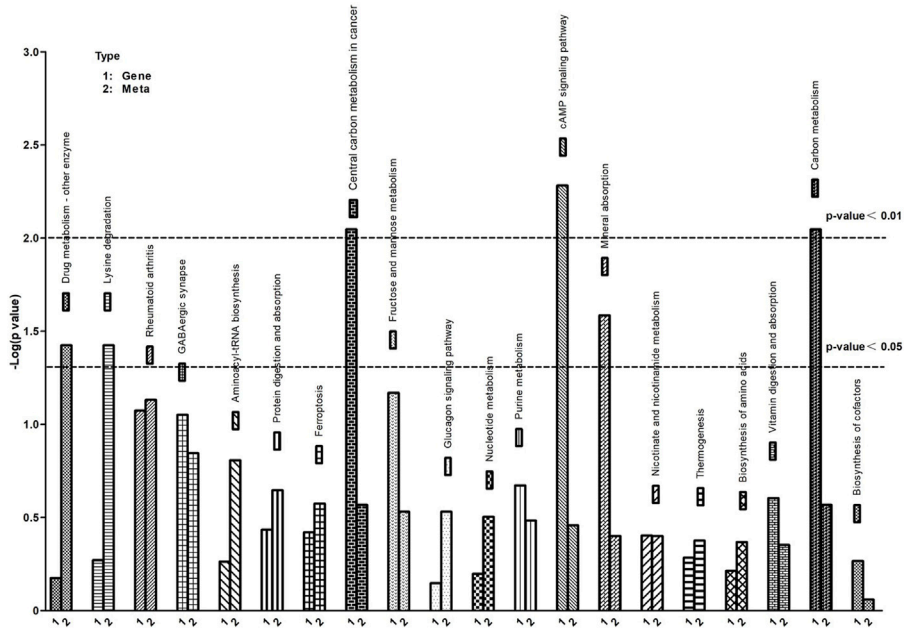


FIGURE 7
KEGG pathway enrichment of differential genes and metabolites. The abscissa represents signaling pathways, the ordinate represents enriched *p*-values, expressed as -log (*p*-value).

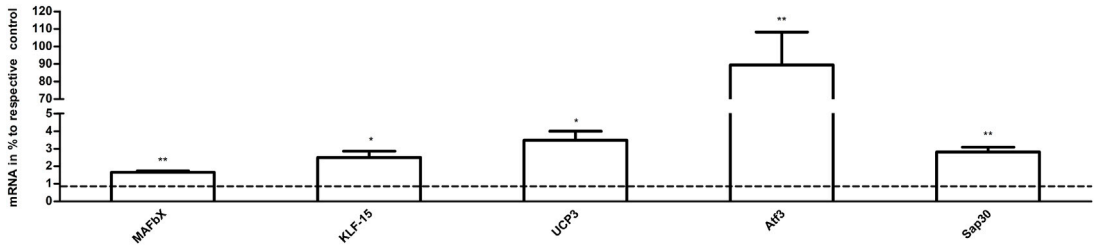


FIGURE 8
The effects of acute exercise on the gene expression of muscle. Compared with the control group, the mRNA expression in the exercise group. The relative levels of target gene mRNA expression were normalized against the mRNA expression of internal housekeeping gene (β-actin). The expression of *β*-actin mRNA was not significantly different between the tested groups allowing a direct comparison (data not shown). Note that columns and error bars represent (mRNA in % to respective control ±SEM), and levels of significance (**p* < 0.05, ***p* < 0.01, n = 3) are indicated.

system (Cromm and Crews, 2017). There is evidence that abnormal protein metabolism can lead to excessive activation of the ubiquitin-proteasome system, resulting in protein degradation (Cunha et al., 2012). MAFbx and MuRF-1, which are both downstream signaling proteins of FoxO, are widely recognized as crucial skeletal muscle-specific F-box-type ubiquitin ligases involved in protein ubiquitination (Bodine and Baehr, 2014). This study demonstrates an enhanced process of ubiquitin mediated proteolysis, as depicted in Figure 6. Previous studies have examined the expression of MAFbx and MuRF1 genes in skeletal muscle during exercise. Mascher et al. (2008) found that after one session of knee joint resistance exercise in sedentary young men, the mRNA content of MuRF1 increased by 4.8 times. Louis et al. (2007) had subjects perform running and resistance exercises and found increased expression of MuRF1 and MAFbx through a muscle biopsy.

UPS could regulate the degradation of proteins involved in iron metabolism, thereby modulating ferroptosis (Zhang et al., 2010; Horie et al., 2017). In this study, Figure 7 vividly depicts the crucial discovery that ferroptosis serves as a pivotal biological process, identified through KEGG pathway enrichment analysis of differential genes and metabolites. Ferroptosis, a novel type of cell death has been reported (Dixon et al., 2012). It is a specific mechanism characterized by lipid peroxidation due to iron overload, which leads to cell death (Conrad et al., 2016). Studies have shown that ferroptosis is associated with various cellular processes, such as iron homeostasis, redox homeostasis, and lipid metabolism (Yang et al., 2014; Ingold et al., 2018). UPS, as a key regulatory system for homeostasis of eukaryotic cells (Glickman and Ciechanover, 2002). Ferroptosis impacted muscle fiber differentiation and promoted muscle protein degradation.

Interestingly, some ubiquitin ligases are also involved in ferroptosis occurrence (Meng et al., 2022), while myasthenia-specific MAFbx and MuRF1 expression increases with iron accumulation in the skeletal muscle of aging individuals (Bodine and Baehr, 2014; Huang et al., 2021).

Thus, take all together, this study identified multiple metabolic pathways and signaling pathways that were involved in the regulation of skeletal muscle, such as the TCA cycle, oxidative phosphorylation, protein digestion and absorption, the FoxO signaling pathway, ubiquitin mediated proteolysis, and ferroptosis signaling pathway. Additionally, the study demonstrated that acute exercise led to the upregulation of KLF-15, FoxO1, MAFbx, and MuRF1 expression, which could play a critical role in enhancing skeletal muscle proteolysis. However, the study mainly focused on the metabolic and transcription profiling of mouse muscle, and did not involve other potential biomarkers or mechanisms, such as protein spectrometry or post-transcriptional modifications of gene expression.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/search/all/?term=PRJNA1002978.

Ethics statement

The animal study was approved by Academic Integrity and Research Ethics Committee of China University of Geosciences (Wuhan). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

XY: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing–original draft, Writing–review and editing. RL: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing–original draft, Writing–review and editing. ZQ: Methodology, Writing–review and editing. XC: Methodology, Writing–review and editing. YW: Methodology, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by grants from the Fundamental Research Funds for the Central Universities in China (Grant No. CUG150607).

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.

Publisher's note

References

Baskin, K. K., Winders, B. R., and Olson, E. N. (2015). Muscle as a "mediator" of systemic metabolism. *Cell Metab.* 21, 237–248. doi:10.1016/j.cmet.2014.12.021

Bodine, S. C., and Baehr, L. M. (2014). Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. *Am. J. Physiology-Endocrinology Metabolism* 307, E469–E484. doi:10.1152/ajpendo.00204.2014

Bodine, S. C., Latres, E., Baumhueter, S., Lai, V. K., Nunez, L., Clarke, B. A., et al. (2001). Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* 294, 1704–1708. doi:10.1126/science.1065874

Camera, D. M., Smiles, W. J., and Hawley, J. A. (2016). Exercise-induced skeletal muscle signaling pathways and human athletic performance. *Free Radic. Biol. Med.* 98, 131–143. doi:10.1016/j.freeradbiomed.2016.02.007

Castellanos, N., Diez, G. G., Antúnez-Almagro, C., Bailén, M., Bressa, C., González Soltero, R., et al. (2020). A critical mutualism–competition interplay underlies the loss of microbial diversity in sedentary lifestyle. *Front. Microbiol.* 10, 3142. doi:10.3389/fmicb.2019.03142

Conrad, M., Angeli, J. P. F., Vandenabeele, P., and Stockwell, B. R. (2016). Regulated necrosis: disease relevance and therapeutic opportunities. *Nat. Rev. Drug Discov.* 15, 348–366. doi:10.1038/nrd.2015.6

Cook, D. B., Stegner, A. J., Nagelkirk, P. R., Meyer, J. D., Togo, F., and Natelson, B. H. J. M. (2012). Responses to exercise differ for chronic fatigue syndrome patients with fibromyalgia. *Med. Sci. sports Exerc.* 44, 1186–1193. doi:10.1249/MSS.0b013e3182417b9a

Cromm, P. M., and Crews, C. M. (2017). The proteasome in modern drug discovery: second life of a highly valuable drug target. *ACS Cent. Sci.* 3, 830–838. doi:10.1021/acscentsci.7b00252

Crow, M. T. (2002). Hypoxia, BNip3 proteins, and the mitochondrial death pathway in cardiomyocytes. *Circ. Res.* 91, 183–185. doi:10.1161/01.res.0000030195.38795.cf

Cunha, T. F., Bacurau, A. V., Moreira, J. B., Paixao, N. A., Campos, J. C., Ferreira, J. C., et al. (2012). Exercise training prevents oxidative stress and ubiquitin-proteasome system overactivity and reverse skeletal muscle atrophy in heart failure. *PLoS One* 7, e41701. doi:10.1371/journal.pone.0041701

Defronzo, R. A., and Tripathy, D. (2009). Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* 32 (2), S157–S163. doi:10.2337/dc09-S302

Dettmer, K., Aronov, P. A., and Hammock, B. D. (2007). Mass spectrometry-based metabolomics. *Mass Spectrom. Rev.* 26, 51–78. doi:10.1002/mas.20108

Dixon, S. J., Lemberg, K. M., Lamprecht, M. R., Skouta, R., Zaitsev, E. M., Gleason, C. E., et al.(2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 149, 1060–1072. doi:10.1016/j.cell.2012.03.042

Dodd, S. L., Gagnon, B. J., Senf, S. M., Hain, B. A., and Judge, A. R. (2010). Ros-mediated activation of NF-kappaB and Foxo during muscle disuse. *Muscle Nerve* 41, 110–113. doi:10.1002/mus.21526

Dohm, G. L., Kasperek, G. J., Tapscott, E. B., and Barakat, H. A. (1985). Protein metabolism during endurance exercise. *Fed. Proc.* 44, 348–352.

Egan, B., and Zierath, J. R. (2013). Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metab.* 17, 162–184. doi:10.1016/j.cmet.2012.12.012

Fan, W., and Evans, R. M. (2017). Exercise mimetics: impact on health and performance. *Cell Metab.* 25, 242–247. doi:10.1016/j.cmet.2016.10.022

Farr, J. N., and Khosla, S. (2019). Cellular senescence in bone. *bone* 121, 121–133. doi:10.1016/j.bone.2019.01.015

Glickman, M. H., and Ciechanover, A. J. P. R. (2002). The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol. Rev.* 82, 373–428. doi:10.1152/physrev.00027.2001

Gomes, M. D., Lecker, S. H., Jagoe, R. T., Navon, A., and Goldberg, A. L. (2001). Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *Proc. Natl. Acad. Sci. U. S. A.* 98, 14440–14445. doi:10.1073/pnas.251541198

Harris, R. A., Joshi, M., Jeoung, N. H., and Obayashi, M. (2005). Overview of the molecular and biochemical basis of branched-chain amino acid catabolism. *J. Nutr.* 135, 1527S–1530S. doi:10.1093/jn/135.6.1527S

Hochachka, P. W., and Dressendorfer, R. H. (1976). Succinate accumulation in man during exercise. *Eur. J. Appl. Physiol. Occup. Physiol.* 35, 235–242. doi:10.1007/BF00423282

Horie, T., Kawamata, T., Matsunami, M., and Ohsumi, Y. (2017). Recycling of iron via autophagy is critical for the transition from glycolytic to respiratory growth. *J. Biol. Chem.* 292, 8533–8543. doi:10.1074/jbc.M116.762963

Huang, Y., Wu, B., Shen, D., Chen, J., Yu, Z., and Chen, C. (2021). Family function fully mediates the relationship between social support and perinatal depression in rural Southwest China. *Int. J. Biol. Sci.* 17, 151. doi:10.1186/s12888-021-03155-9

Ingold, I., Berndt, C., Schmitt, S., Doll, S., Poschmann, G., Buday, K., et al.(2018). Selenium utilization by GPX4 is required to prevent hydroperoxide-induced ferroptosis. *Cell* 172, 409–422. doi:10.1016/j.cell.2017.11.048

Kemi, O. J., Loennechen, J. P., Wisloff, U., and Ellingsen, O. (2002). Intensity-controlled treadmill running in mice: cardiac and skeletal muscle hypertrophy. *J. Appl. Physiol.* 93, 1301–1309. doi:10.1152/japplphysiol.00231.2002

Kim, S., Hong, J., Jeon, R., and Kim, H.-S. (2016). Adzuki bean ameliorates hepatic lipogenesis and proinflammatory mediator expression in mice fed a high-cholesterol and high-fat diet to induce nonalcoholic fatty liver disease. *Nutr. Res.* 36, 90–100. doi:10.1016/j.nutres.2015.11.002

Kruger, K., Frost, S., Most, E., Volker, K., Pallauf, J., and Mooren, F. C. (2009). Exercise affects tissue lymphocyte apoptosis via redox-sensitive and Fas-dependent signaling pathways. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R1518–R1527. doi:10.1152/ajpregu.90994.2008

Kuo, T., Harris, C. A., and Wang, J.-C. (2013). Metabolic functions of glucocorticoid receptor in skeletal muscle. *Mol. Cell. Endocrinol.* 380, 79–88. doi:10.1016/j.mce.2013.03.003

Langmead, B., and Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9, 357–359. doi:10.1038/nmeth.1923

Li, B., and Dewey, C. N. (2011). RSEM: accurate transcript quantification from RNA-seq data with or without a reference genome. *BMC Bioinforma.* 12, 323. doi:10.1186/1471-2105-12-323

Louis, E., Raue, U., Yang, Y., Jemiolo, B., and Trappe, S. (2007). Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. *J. Appl. physiology* 103, 1744–1751. doi:10.1152/japplphysiol.00679.2007

Magalhaes, J., Fraga, M., Lumini-Oliveira, J., Goncalves, I., Costa, M., Ferreira, R., et al. (2013). Eccentric exercise transiently affects mice skeletal muscle mitochondrial function. *Appl. Physiol. Nutr. Metab.* 38, 401–409. doi:10.1139/apnm-2012-0226

Mascher, H., Tannerstedt, J., Brink-Elfegoun, T., Ekblom, B., Gustafsson, T., and Blomstrand, E. (2008). Repeated resistance exercise training induces different changes in mRNA expression of MAFbx and MuRF-1 in human skeletal muscle. *Am. J. Physiology Endocrinol. Metabolism* 294, E43–E51. doi:10.1152/ajpendo.00504.2007

Meng, J., Zou, X., Wu, R., Zhong, R., Zhu, D., and Zhang, Y. (2014). Accelerated regeneration of the skeletal muscle in RNF13-knockout mice is mediated by macrophage-secreted IL-4/IL-6. *Protein and Cell* 5, 235–247. doi:10.1007/s13238-014-0025-4

Meng, Y., Sun, H., Li, Y., Zhao, S., Su, J., Zeng, F., et al. (2022). Targeting ferroptosis by ubiquitin system enzymes: a potential therapeutic strategy in cancer. *Int. J. Biol. Sci.* 18, 5475–5488. doi:10.7150/ijbs.73790

Mokranjac, D., Bourenkov, G., Hell, K., Neupert, W., and Groll, M. (2006). Structure and function of Tim14 and Tim16, the J and J-like components of the mitochondrial protein import motor. *EMBO J.* 25, 4675–4685. doi:10.1038/sj.emboj.7601334

Mosser, D. D., Caron, A. W., Bourget, L., Meriin, A. B., Sherman, M. Y., Morimoto, R. I., et al. (2000). The chaperone function of hsp70 is required for protection against stress-induced apoptosis. *Mol. Cell. Biol.* 20, 7146–7159. doi:10.1128/mcb.20.19.7146-7159.2000

Nabben, M., Hoeks, J., Briedé, J. J., Glatz, J. F., Moonen-Kornips, E., Hesselink, M. K., et al. (2008). The effect of UCP3 overexpression on mitochondrial ROS production in skeletal muscle of young versus aged mice. *FEBS Lett.* 582, 4147–4152. doi:10.1016/j.febslet.2008.11.016

Naseer, M. I., Abdulkareem, A. A., Jan, M. M., Chaudhary, A. G., Alharazy, S., and Alqahtani, M. H. (2020). Next generation sequencing reveals novel homozygous frameshift in PUS7 and splice acceptor variants in AASS gene leading to intellectual disability, developmental delay, dysmorphic feature and microcephaly. *Saudi J. Biol. Sci.* 27, 3125–3131. doi:10.1016/j.sjbs.2020.09.033

O'neill, C. A., Stebbins, C. L., Bonigut, S., Halliwell, B., and Longhurst, J. C. (1996). Production of hydroxyl radicals in contracting skeletal muscle of cats. *J. Appl. Physiol.* 81, 1197–1206. doi:10.1152/jappl.1996.81.3.1197

Puthanveetil, P., Wan, A., and Rodrigues, B. (2013). FoxO1 is crucial for sustaining cardiomyocyte metabolism and cell survival. *Cardiovasc Res.* 97, 393–403. doi:10.1093/cvr/cvs426

Qiu, S. T., Cui, D., Lu, J., and Chen, C. Z. (2014). Effect of AMPK/Sirt1 pathway on the muscle mass control induced by exercise. *J. Tianjin Univ. Sport* 27, 255–258.

Reid, M. B., Haack, K. E., Franchek, K. M., Valberg, P. A., Kobzik, L., and West, M. S. (1992). Reactive oxygen in skeletal muscle. I. Intracellular oxidant kinetics and fatigue *in vitro*. *J. Appl. Physiol.* 73, 1797–1804. doi:10.1152/jappl.1992.73.5.1797

Rennie, M. J., Edwards, R. H., Davies, C. T., Krywawych, S., Halliday, D., Waterlow, J. C., et al. (1980). Protein and amino acid turnover during and after exercise. *Biochem. Soc. Trans.* 8, 499–501. doi:10.1042/bst0080499

Samant, S. A., Kanwal, A., Pillai, V. B., Bao, R., and Gupta, M. P. (2017). The histone deacetylase SIRT6 blocks myostatin expression and development of muscle atrophy. *Sci. Rep.* 7, 11877. doi:10.1038/s41598-017-10838-5

Schoepe, M., Schrepper, A., Schwarzer, M., Osterholt, M., and Doenst, T. (2012). Exercise can induce temporary mitochondrial and contractile dysfunction linked to impaired respiratory chain complex activity. *Metabolism-clinical Exp.* 61, 117–126. doi:10.1016/j.metabol.2011.05.023

Shen, F., Zhao, Y., Ding, W., Liu, K., Ren, X., Zhang, Q., et al. (2021). Autonomous climbing: an effective exercise mode with beneficial outcomes of aerobic exercise and resistance training. *Life Sci.* 265, 118786. doi:10.1016/j.lfs.2020.118786

Song, Y., Shi, X., Gao, Z., Li, R., Tian, J., Cao, X., et al. (2023). Acupoint catgut embedding improves lipid metabolism in exercise-induced fatigue rats via the PPAR signaling pathway. *Anim. (Basel)* 13, 558. doi:10.3390/ani13040558

Toime, L. J., and Brand, M. D. (2010). Uncoupling protein-3 lowers reactive oxygen species production in isolated mitochondria. *Free Radic. Biol. Med.* 49, 606–611. doi:10.1016/j.freeradbiomed.2010.05.010

Tondera, D., Grandemange, S., Jourdain, A., Karbowski, M., Mattenberger, Y., Herzig, S., et al.(2009). SLP-2 is required for stress-induced mitochondrial hyperfusion. *EMBO J.* 28, 1589–1600. doi:10.1038/emboj.2009.89

Vezzoli, A., Dellanoce, C., Mrakic-Sposta, S., Montorsi, M., Moretti, S., Tonini, A., et al. (2016). Oxidative stress assessment in response to ultraendurance exercise: thiols redox status and ROS production according to duration of a competitive race. *Oxid. Med. Cell Longev.* 2016, 6439037. doi:10.1155/2016/6439037

Wang, F., Wang, X., Liu, Y., and Zhang, Z. (2021). Effects of exercise-induced ROS on the pathophysiological functions of skeletal muscle. *Oxidative Med. Cell. Longev.* 2021, 3846122–3846125. doi:10.1155/2021/3846122

Wang, Z., Gerstein, M., and Snyder, M. (2009). RNA-seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.* 10, 57–63. doi:10.1038/nrg2484

Yang, W. S., Sriramaratnam, R., Welsch, M. E., Shimada, K., Skouta, R., Viswanathan, V. S., et al.(2014). Regulation of ferroptotic cancer cell death by GPX4. *Cell* 156, 317–331. doi:10.1016/j.cell.2013.12.010

Zhang, Y., Mikhael, M., Xu, D., Li, Y., Soe-Lin, S., Ning, B., et al. (2010). Lysosomal proteolysis is the primary degradation pathway for cytosolic ferritin and cytosolic ferritin degradation is necessary for iron exit. *Antioxidants redox Signal.* 13, 999–1009. doi:10.1089/ars.2010.3129

Zhao, T., Mu, X., and You, Q. (2017). Succinate: an initiator in tumorigenesis and progression. *Oncotarget* 8, 53819–53828. doi:10.18632/oncotarget.17734

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 15 November 2023
DOI 10.3389/fphys.2023.1269885

OPEN ACCESS

EDITED BY
Simone Luti,
University of Florence, Italy

REVIEWED BY
Antonella Muscella,
University of Salento, Italy
Gerasimos V. Grivas,
Hellenic Naval Academy, Greece

*CORRESPONDENCE
Su Meihua,
sumh1234@163.com

RECEIVED 31 July 2023
ACCEPTED 06 November 2023
PUBLISHED 15 November 2023

CITATION
Meihua S, Jiahui J, Yujia L, Shuang Z and Jingjing Z (2023), Research on sweat metabolomics of athlete's fatigue induced by high intensity interval training. *Front. Physiol.* 14:1269885. doi: 10.3389/fphys.2023.1269885

Research on sweat metabolomics of athlete's fatigue induced by high intensity interval training

Su Meihua[1]*, Jin Jiahui[1], Li Yujia[1], Zhao Shuang[2] and Zhan Jingjing[2]

[1]School of Physical Education, Jimei University, Xiamen, Fujian, China, [2]Xiamen Meliomics Technology Co., Ltd., Xiamen, Fujian, China

Objective: Sweat is an important specimen of human metabolism, which can simply and non-invasively monitor the metabolic state of the body, and its metabolites can be used as biomarkers for disease diagnosis, while the changes of sweat metabolites before and after exercise-induced fatigue are still unclear.

Methods: In this experiment, high-performance chemical isotope labeling liquid chromatography-mass spectrometry (LC-MS) was used to metabolomic 28 sweat samples before and after exercise-induced fatigue of 14 long-distance runners, also IsoMS PRO and SPSS22.0 software were used to analyze the metabolite changes and differential metabolic pathways.

Results: A total of 446 metabolites with high confidence were identified, and the sweat metabolome group before and after high-intensity interval exercise-induced fatigue was obvious, among which the upregulated differential metabolites mainly included hypoxanthine, pyruvate, several amino acids, etc., while the downregulated differential metabolites mainly included amino acid derivatives, vitamin B6, theophylline, etc.

Conclusion: The change of hypoxanthine concentration in sweat can be used as a good biomarker for the diagnosis of exercise-induced fatigue, while the change of pyruvate content in sweat can be used as a discriminant index for the energy metabolism mode of the body before and after exercise. The main metabolic pathways involved in differential metabolites produced before and after HIIT exercise-induced fatigue are purine metabolism and amino acid metabolism.

KEYWORDS

metabolomics, exercise-induced fatigue, human sweat, high-intensity interval training, high performance chemical isotope labeling liquid chromatography-tandem mass spectrometry

1 Introduction

Organisms are stable systems, even as molecules in the organism are constantly changing in countless chemical reactions. Based on the Greek word for change, "metabolism" is used to describe all chemical reactions in an organism that change molecules. Exercise alters the concentration of many metabolites, small molecules (<1.5 kDa) metabolized by the body's metabolic reaction. Sweat is a colorless, hypotonic solution produced by the sweat glands within the epidermis of the body. The main components of sweat include water, electrolytes (sodium, potassium and chloride), urea, pyruvate, lactate and amino acids, along with

proteins, peptides, drugs and other exogenous substances, and its main function is to regulate body temperature by evaporating to cool the body in response to high temperatures or physical exercise. Studies have shown that sweat gland proteomics is significantly different from serum proteomics and that sweat components are not only diffusible fluids from plasma, but may represent surrounding tissue and cellular metabolic processes (Hooton et al., 2016; Schranner et al., 2020; Delgado-Povedano et al., 2018). Exercise-induced fatigue refers to the inability to sustain its function at a specific level and/or to maintain a predetermined intensity of exercise. Exercise-induced fatigue could reduce reaction ability and exercise performance, and lead to safety risks, so it is very important to find effective indicators for monitoring the occurrence of exercise-induced fatigue and improve the sports performance of athlete (Meeusen et al., 2021). Metabolomics currently monitors the disease and metabolic status of the human body by detecting trace changes in biological samples such as urine, blood and saliva, which is difficult to achieve with traditional detection and analysis techniques, and has been widely used in several disciplines due to its systematic, comprehensive and high-throughput advantages (Cai et al., 2022). There are a total of 1.6–4 million sweat glands on most of the human epidermis, and most sweat components are small molecules (<1,000 Da), making sweat sampling a non-invasive and non-invasive method compared to other bodily fluids such as serum, and allowing easy regular or continuous collection of observables (Cai et al., 2022). Therefore, sweat specimens have great potential to be mined for disease biomarkers and allow early monitoring of different physiological states (Hooton et al., 2016; Schranner et al., 2020). Sweat is currently less used in metabolomics and the metabolites in it are difficult to detect, but data reports on changes of sweat metabolites after exercise-induced fatigue are still relatively limited. Therefore, how the fatigue status of the body after high-intensity exercise in athletes is reflected by sweat metabolites may be a new idea to explore to delay the onset of exercise-induced fatigue and to conveniently monitor the functional status of athletes. In this paper, the metabolomic changes of sweat before and after high-intensity intermittent exercise were selected to observe the changes of body metabolites in sweat before and after exercise-induced fatigue, in an attempt to find metabolic markers for early diagnosis of exercise-induced fatigue through convenient and non-invasive sweat, and to provide a reference basis for delaying the onset of fatigue and preventing sports injuries.

2 Sweat sampling and analysis

2.1 Sweat sampling

Fourteen junior high school long-distance runners (7 male and 7 female), who usually live in school and work with the same coach, with a training period of 2–4 years, were selected to meet the inclusion criteria of this experiment: ① no history of special diseases; ② no smoking and drinking habits; ③ no special drugs or physical discomfort before training. All participating subjects were informed of the test procedure and purpose, and signed the informed consent form. The experimental protocol and experimental procedure were approved and supervised by the Academic and Ethical Ethics Committee of Jimei University.

2.2 Exercise protocol of the subjects

The changes in sweat metabolomics of the athletes before and after training were observed by selecting one of their training sessions, and the testing site was the 400-m track and field playground of the junior high school. The warming-up exercises of this long-distance runners consisted of jogging, press-kick, trot, high leg lift, and back stomp running, which takes about 18 min. The formal exercise is usually used by coaches to improve the speed endurance of athletes, which is intermittent high-intensity training (Manoel et al., 2022). This intermittent high-intensity training includes four 100-m runs with a 2-min interval and a 4-min rest after the 100-m run, which took about 12 min; followed by four 400-m all-out runs with a 4-min interval and a 5-min rest between groups, which took about 25 min; Finally, an 800-m run, which took about 3 min. Sweat patches were removed immediately after the 800-m run, so the total sweat collection time for the formal exercise was about 40 min. The time of every trial of each athlete's 100-m and 400-m runs was recorded, and the best test scores of each athlete's 100-m and 400-m runs was chosen for the further statistical analysis. The basic information of the athletes and the test scores of that training session are shown in Table 1.

2.3 Collection of sweat

Patches of sweat were fabricated according to the literature (Hooton et al., 2016), as shown in Figure 1. The sweat collection area was first wiped with dust-free paper (Kimwipe) soaked in 70% isopropyl alcohol-water to clean the sampling area and dry naturally. The protective layer of the transparent film dressing (Tegaderm, 6 cm × 7 cm) was removed and subsequently placed on a flat surface with the adhesive side facing upwards. Two clean filter papers (Whatman) with a diameter of 1.5 cm are attached to the Tegaderm dressing approximately 4 cm apart. Then, two clean filter papers of 1.27 cm diameter are carefully placed on the collection area (approximately 4 cm apart). Finally, the Tegaderm dressing is secured to the 1.27 cm filter paper in the collection area and ensures that the filter paper is aligned over the adhesive and skin. Two pieces of filter paper are applied to the forearm before the athlete prepares for the activity to collect pre-exercise sweat. At the end of the preparation activity, the athletes' sweat filter papers were removed separately and placed in a zip plastic bag, then placed the zip bags in an ice pack for about 20 min, and then quickly put the zip bags in a box and stored them at −80°C in the refrigerator to be measured. Before the start of the formal exercise, new filter papers were quickly applied to the same parts of the athlete and sweat was collected from the start of the formal exercise until the end of the exercise.

2.4 Determination of exercise-induced fatigue

Determination of exercise-induced fatigue: According to the criteria for determining exercise-induced fatigue (Khoramipour et al., 2021), the athletes' post-exercise RPE values reached 17-19, and the athletes' urine protein was negative in the morning,

TABLE 1 Basic information of the athletes and the test results of the training class.

Gender	Age (years)	Height (cm)	Weight (kg)	The 100-m score(s)	The 400-m score(s)	The 800-m score(s)
Male	16.33 ± 1.93	175.78 ± 5.95	61.00 ± 4.21	11.87 ± 0.83	54.94 ± 2.52	126.00 ± 3.16
Female	16.75 ± 1.75	161.13 ± 4.39	46.63 ± 5.47	13.71 ± 0.34	63.47 ± 1.50	150.75 ± 3.77

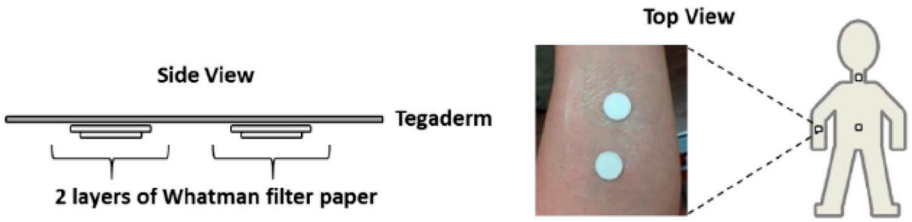


FIGURE 1
Fabrication diagram of sweat patch.

positive 3 h after exercise and negative in the morning of the next day. In this experiment, by measuring the post-training RPE scale and the corresponding heart rate intensity of the long-distance runners, the average subjective fatigue perception of the athletes was 17.84 ± 0.55, and all the athletes were negative for urine protein in the morning, positive for urine protein 3 h after exercise, and negative for urine protein in the next morning. Therefore, all the subjects in this experiment had achieved exercise-induced fatigue after training.

2.5 Sample labeling

Sample preparation was performed using the Dansylamide labeling kit (Hooton et al., 2016). A number of 2 mL centrifuge tubes with filters were prepared, and the sweat patches were cut into small pieces with medical scissors and placed in the filters of the centrifuge tubes with filters. 50 μL of mass Pierce™ water was added, and the samples were allowed to stand for 5 min at room temperature, followed by high speed centrifugation (13,000 rpm, 5 min) at 4°C. Each sample was divided into 2 fractions for channel analysis (25 μL/channel) and mixed sample preparation. For split samples for amine/phenolic secondary metabolome analysis, samples were evaporated dry using a nitrogen concentrator, then 25 μL of mass Pierce™ water was added to re-dissolve the samples and sample labeling was performed in strict accordance with standard operating procedures (SOPs) and kit requirements. First, 12.5 μL of sodium bicarbonate buffer is added to the sample, followed by 37.5 μL of 12C-labeled Dansylamide chloride solution, followed by vortex mixing and incubation at 40°C for 45 min. After incubation, 7.5 μL of sodium hydroxide solution is added to quench the excess labeling reagent and the mixture is incubated at 40°C for 10 min. Finally, 30 μL of formic acid solution is added.

2.6 Sample mixing and LC-UV analysis conditions

The labeled amine/phenol secondary metabolome was quantified using LC-UV according to standard operating procedures (Schranner et al., 2020). Based on the quantification results, equal amounts of 13C-labeled mixed samples are added to the 12C-labeled individual samples for liquid-liquid analysis. Before the liquid-liquid analysis, quality control samples were also prepared by mixing equal volumes of 13C-labeled samples and 12C-labeled samples as quality control samples. All samples were prepared and analyzed according to the standard procedure.

2.7 Data processing

A total of 31 data (including 28 data samples and 3 quality control sample data) were collected. Data was analyzed using IsoMS Pro, software and the NovaMT Metabolomics database (Nova Medical Testing Inc., Canada). Data before and after exercise-induced fatigue were analyzed by paired sample *t*-test by SPSS 22.0 software, data were expressed as mean ± SD, both $p < 0.05$ and $p < 0.001$ were scored as significant.

3 Results

3.1 Data quality control

In the amine/phenol channel analysis, the peak with a mass-to-charge ratio (m/z) of 251.0849 was selected as the background peak for the quality accuracy check of the data from 31 samples, as shown in Figure 2. The mass-to-charge ratios of all scans were within the expected range, indicating good stability and quality accuracy of the collected data.

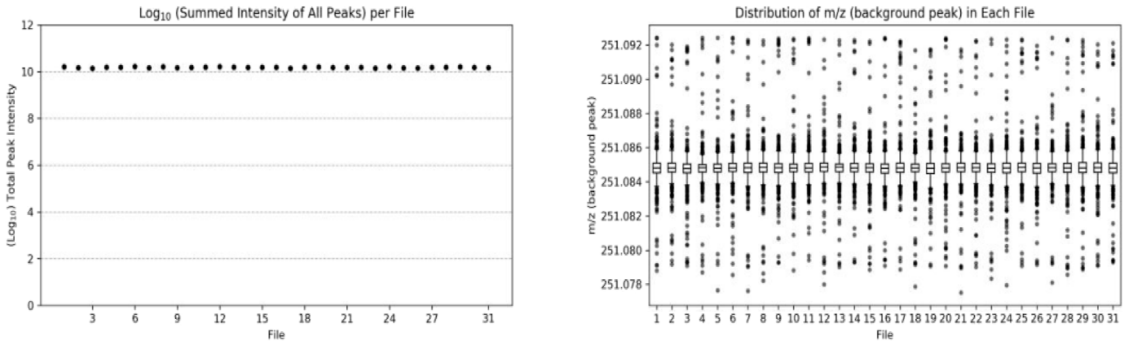


FIGURE 2
Number (left) and distribution (right) of background peaks in the analysis of amine/phenol-based channel samples.

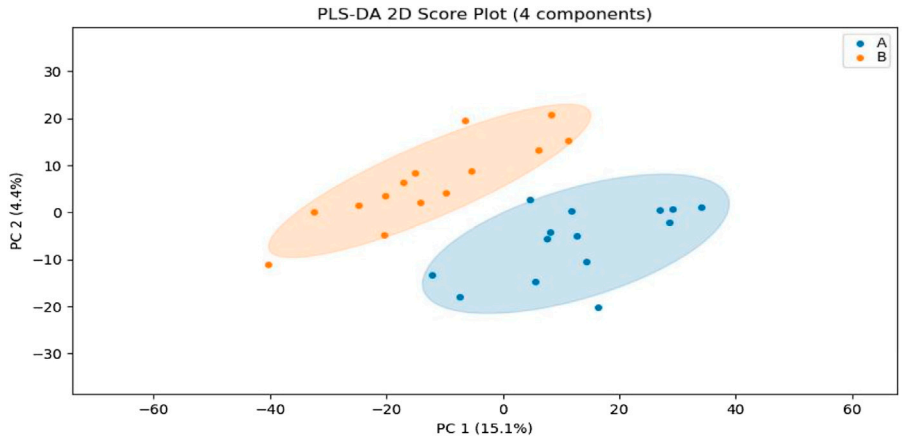


FIGURE 3
PLS-DA analysis of sweat metabolism before and after exercise-induced fatigue. Note: A is the sweat sample before exercise-induced fatigue; B is the sweat sample after exercise-induced fatigue.

3.2 Partial least squares discriminant analysis of sweat metabolism pre and post exercise-induced fatigue

In this experiment, sweat metabolism before and after exercise-induced fatigue was analyzed by using Partial least squares Discriminant Analysis (PLS-DA), as shown in Figure 3, the metabolites grouped significantly before and after exercise-induced fatigue in long-distance runners, indicating that the physiological state of long-distance runners before and after exercise-induced fatigue at the metabolic level was significantly different ($p < 0.05$).

3.3 Volcano plot analysis of sweat metabolism in long-distance runners before and after exercise-induced fatigue

In this experiment, volcanoes were plotted by using difference multiples (FC) and p-values, as shown in Figure 4. When using difference multiples >1.2 or <0.83 and $p < 0.05$ as the criteria for differential metabolites, the corresponding Storey's q value (i.e., FDR-adjusted p-value) threshold was 0.0487, and the analysis showed that a total of 439 metabolites met FC > 1.2 and $p < 0.05$, and 139 metabolites met FC < 0.83 and $p < 0.05$. As seen in Figure 4, 129 metabolites were significantly downregulated and 401 metabolites were significantly upregulated after exercise-induced fatigue in long-distance runners. Of these, 446 metabolites were identified by methods with high confidence (via CIL Library and LI Library databases) and these can be used for further metabolic pathway analysis.

3.4 Analysis of differential metabolites in sweat of long-distance runners before and after exercise-induced fatigue

Differential metabolites of sweat before and after exercise were obtained by using metabolites from the CIL Library (CIL) and LI Library (LI) databases, and again using Fold Change (FC), i.e., FC > 1.5 or FC < 0.67, t-test probability p-value less than 0.02 and contribution value (variable importance in the projection, VIP)

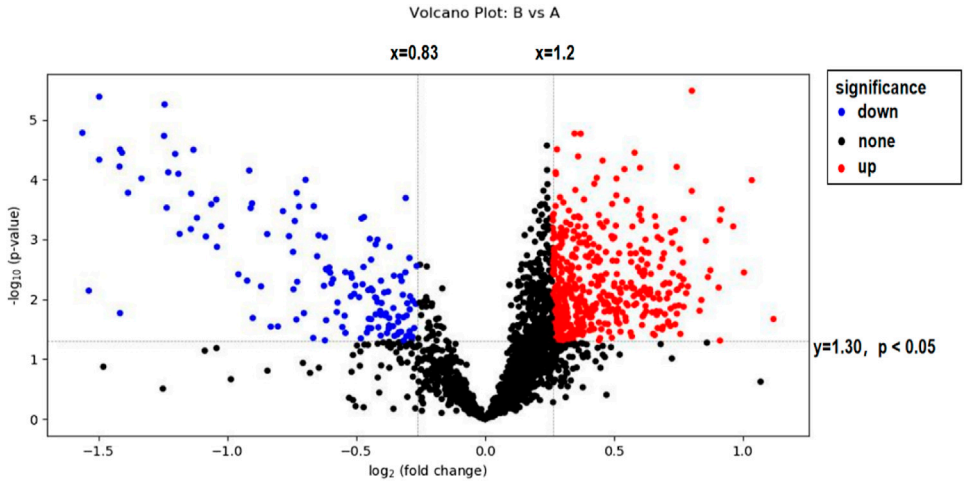


FIGURE 4
Volcano map analysis of sweat metabolites before and after exercise-induced fatigue Note: A is the sweat sample before exercise-induced fatigue; B is the sweat sample after exercise-induced fatigue.

TABLE 2 Differential metabolites of sweat pre and post exercise-induced fatigue in long-distance runners.

NO.	Name of metabolite	Number	Metabolite molecular formula	*p*-value	VIP	Difference multiplier	Variation
1	Hypoxanthine	C00262	$C_5H_4N_4O$	0.0009	2.624	1.5912	↑
2	Methionyl-Serine	HMDB0028982	$C_8H_{16}N_2O_4S$	0.0003	1.663	1.5141	↑
3	N-Carboxyethyl-g-aminobutyric acid	HMDB0002201	$C_7H_{13}NO_4$	0.0001	1.462	1.7439	↑
4	Phenylalanyl-Asparagine	HMDB0028990	$C_{13}H_{17}N_3O_4$	0.0003	1.441	1.5198	↑
5	N-Methyl-L-glutamic acid	C01046	$C_6H_{11}NO_4$	0.0005	1.324	1.5842	↑
6	D-1-Amino-2-pyrrolidinecarboxylic acid	HMDB0030405	$C_5H_{10}N_2O_2$	0.0068	1.262	1.5584	↑
7	Porphobilinogen	C00931	$C_{10}H_{14}N_2O_4$	0.0096	1.217	1.6312	↑
8	Salsoline-1-carboxylic acid	HMDB0013067	$C_{12}H_{15}NO_4$	0.0079	1.149	1.5062	↑
9	Glutaminylalanine	HMDB0028790	$C_8H_{15}N_3O_4$	0.0037	1.097	1.5347	↑
10	2-Hydroxy-2,4-pentadienoic acid	C00596	$C_5H_6O_3$	0.0002	1.685	0.5325	↓
11	(E)-3-(2-Hydroxyphenyl)-2-propenal	HMDB0031725	$C_9H_8O_2$	0.0031	1.48	0.6520	↓
12	5,6-Dihydroxyindole	C05578	$C_8H_7NO_2$	0.0029	1.367	0.6578	↓
13	N-(6-Aminohexanoyl)-6-aminohexanoic acid	C01255	$C_{12}H_{24}N_2O_3$	0.0006	1.171	0.4533	↓
14	Isomer 1 of Deoxycytidine	C00881	$C_9H_{13}N_3O_4$	0.0038	1.114	0.5151	↓

greater than 1 was used as the screening criteria to obtain the differential metabolites of sweat before and after exercise-induced fatigue in long-distance runners, as shown in Table 2.

As can be seen from Table 2, 14 differential metabolites were screened before and after exercise-induced fatigue in long-distance runners, including 9 upregulated differential metabolites and 5 downregulated differential metabolites, which were hypoxanthine, methionyl-serine, N-carboxyethyl-g-aminobutyric acid, phenylalanyl-asparagine, N-methyl-L-glutamic acid, D-1 amino-2-pyrrolidinecarboxylic acid, cholestyramine, hydrochloride 1-carboxylic acid, and glutamine alanine; while the five downregulated differential metabolisms ranked by VIP value were 2-hydroxy-2,4-pentadienoic acid, (E)-3-(2-hydroxyphenyl)-2-propenoic acid, 5,6-dihydroxyindole, N-(6-aminohexanoyl)-6-aminohexanoic acid, and deoxycytidine isomer 1, in that order.

In this experiment, we further analyzed the top 8 differential metabolites with the smallest *p*-value ranking and VIP greater than 1, as shown in Figure 5. The differential metabolites that passed the CIL Library database and met the minimum *p*-value ranking of the top 8 were Asparagine, Prolyl-Glycine, Phenylalanyl-Asparagine,

10.3389/fphys.2023.1269885

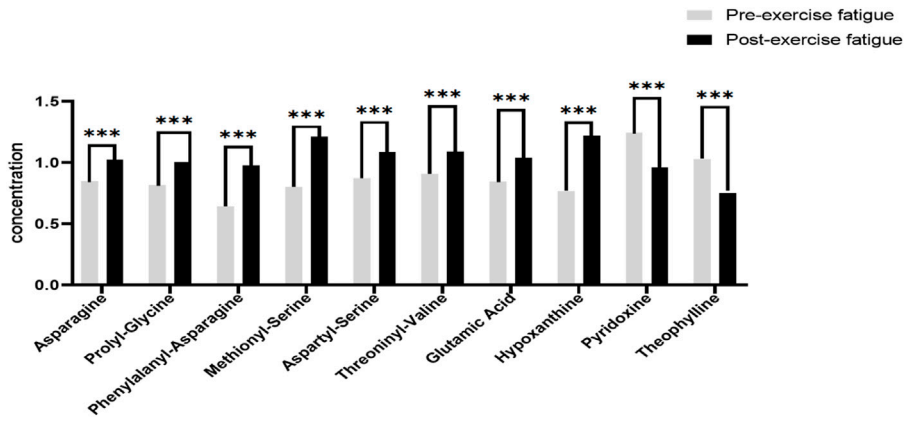


FIGURE 5
Significantly different metabolites of sweat before and after exercise-induced fatigue. Note: Compared with pre-exercise fatigue, *** indicates $p <$ 0.001.

Rank	Identification	VIP Score
1	Hypoxanthine	2.746
2	pyruvic acid	2.727
3	N-(6-Aminohexanoyl)-6-aminohexanoic acid	2.319
4	2'-Aminobiphenyl-2,3-diol	1.856
5	Isomer 1 of 3-Hydroxymandelic acid	1.825
6	4-Hydroxy-4-methylglutamic acid	1.744
7	Isomer 2 of 3-Amino-2-piperidone	1.668
8	1-Phenylethylamine	1.632
9	4-Chloro-L-lysine	1.615
10	(S)-Spinacine	1.613
11	2-Hydroxy-2,4-pentadienoic acid	1.591
12	Isomer 1 of gamma-Amino-gamma-cyanobutanoic acid	1.567
13	3-Amino-2-piperidone	1.559
14	Indoleacrylic acid	1.537
15	Methionyl-Serine	1.536
16	Kanosamine	1.506
17	Isomer 2 of 3,4-Dihydroxystyrene	1.505
18	5-Aminopentanal	1.503
19	2-Aminobenzyl alcohol	1.499
20	Isomer 1 of 2-Dechloroethylifosfamide	1.493

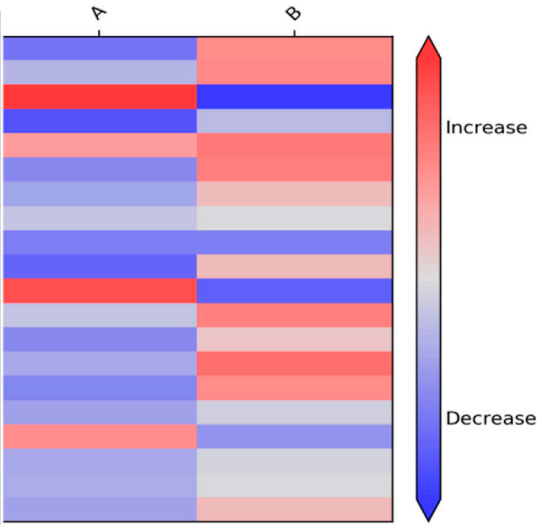


FIGURE 6
Thermogram analysis of metabolites of sweat differences before and after exercise-induced fatigue Note: A is the sweat sample before exercise-induced fatigue; B is the sweat sample after exercise-induced fatigue.

Methionyl-Serine, Aspartyl-Serine, Threoninyl-Valine, Glutamic Acid and Hypoxanthine. All of these 8 differential metabolites showed significant upregulation ($p < 0.001$) after exercise training, while the only 2 differential metabolites that showed significant downregulation ($p < 0.001$) after exercise training through the Cascade 1 database (CIL Library) were Pyridoxine, and Theophylline.

In addition, the differential metabolites that passed through the CIL Library (CIL) and LI Library (LI) databases before and after exercise-induced fatigue and were ranked in the top 10 VIP values are shown by Figure 6, where hypoxanthine and pyruvic acid were ranked 1st and 2nd respectively, and these two metabolites showed significantly increase after this intermittent high-intensity training ($p < 0.001$).

3.5 Analysis of metabolic pathways of sweat before and after exercise-induced fatigue

In this experiment, differential metabolites with high confidence through stratum 1 (CIL Library) and stratum 2 (LI Library) before and after exercise-induced fatigue were selected for metabolic pathway analysis. The analysis was performed using MetaboAnalyst (www.metaboanalyst.ca) Pathway Analysis Module, in which Global Test was used as the enrichment analysis method, Relative-betweeness Centrality as the topological analysis method, and KEGG *Homo sapiens* (human) was used for analysis. In this experiment, $p < 0.05$ and Impact >0.1 were used as screening criteria to obtain the differential metabolic pathways involved in athletes before and after exercise-induced fatigue, as

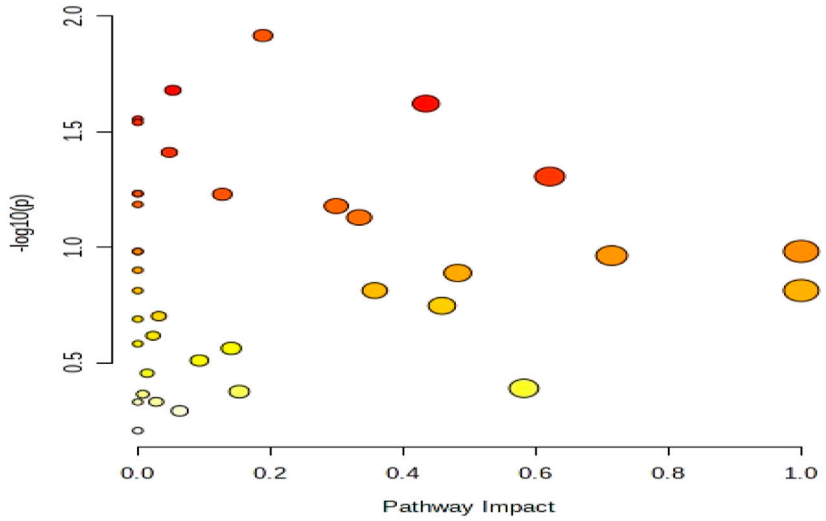


FIGURE 7
Bubble diagram of metabolic pathway analysis of sweat differential metabolites after exercise-induced fatigue.

shown in the bubble diagram of pathway analysis in Figure 7. It is also evident from Figure 7 that the metabolic pathways involved in the major differential metabolites of sweat in long-distance runners after high-intensity interval training (100 m + 400 m + 800 m) are mainly purine metabolism, tryptophan metabolism, alanine, aspartate and glutamate metabolism, and tyrosine metabolism.

4 Discussion and analysis

The field of exercise science has recently begun to apply metabolomics to the study of mechanisms of response and adaptation of body cells, tissues and organs to specific sports (Khoramipour et al., 2021). Sweat, as a mediator connecting the skin barrier, has an irreplaceable role in immune function, skin moisturization, thermoregulation and biodefense (Hooton et al., 2016; Schranner et al., 2020; Delgado-Povedano, 2018). Moreover, due to the non-invasive nature of sweat collection, the monitoring of sweat metabolites in athletes during intense exercise is very important to understand the functional changes of athletes (Souza et al., 2018). Unfortunately, studies on the detection of sweat metabolomics of athletes are still relatively poorly reported. This experiment found that the upregulated differential metabolites produced by long-distance runners after high-intensity interval training mainly included pyruvate, hypoxanthine, and several amino acids, among which the metabolism of alanine and the conversion of pyruvate ensured that glycolysis could continue, suggesting that the athletic training mainly relied on glycolysis for energy supply, while the elevation of hypoxanthine and several amino acids indicated that the body's ATP reserve was mainly relied on ATP reserves and protein reserves for energy after fatigue from high-intensity interval training. This is mainly related to the prolonged intermittent high power and high intensity exercise training performed by the athletes. In addition, the elevation of several amino acids (e.g., glycine, serine) after exercise indicates that the elevated levels of metabolites were associated with oxidative stress (Marinho et al., 2021). Studies have found that several amino acids (e.g., tryptophan, phenylalanine, tyrosine, histidine, valine, leucine, N-acetylaspartate-glutamate, N-acetylaspartate) appear significantly elevated in the urine of national team swimmers after daily training (Sampson et al., 2014). Related studies also found that branched-chain amino acids (BCAAS) involved in glucose regulation such as valine and leucine were significantly increased in male and female rats after forceful exercise (Zhou et al., 2019). These studies are similar to the results of our experiment. Skeletal muscle is a major site utilized by BCAAs (She et al., 2010) and amino acids are also part of the glucose-alanine cycle, which provides glucose to muscle by degrading amino acids, where the remaining amino acids are transported to the liver as alanine to produce ammonia in the urea cycle (María Adeva-Andany et al., 2016). Related studies have also found that fatigued soccer players show significantly increased levels of several amino acids (e.g., valine, isoleucine, tyrosine, leucine, tryptophan, and phenylalanine) and 3-methylhistidine in salivary metabolites after 3 consecutive days of play compared to non-fatigued soccer players (Song-Gyu et al., 2014). Others have found that the concentrations of methionine, valine, and leucine decreased after marathon exercise (Stander et al., 2018). The present experiment also revealed that the downregulated differential metabolites produced after high-intensity intermittent exercise-induced fatigue were mainly amino acid derivatives such as 5,6-dihydroxyindole, N-(6-Aminohexanoyl)-6-aminohexanoic acid, and ketone bodies (E)-3-(2-Hydroxyphenyl)-2-propenal, 2-hydroxy-2,4-pentadienoic acid, and nucleotide metabolites such as Isomer 1 of Deoxycytidine. Recent studies have suggested that different amino acids of the organism change in different directions after exercise (Schranner et al., 2020). Another factor contributing to changes in amino acid concentrations and other metabolites is the pre-exercise dietary regimen; if carbohydrate intake before exercise is inadequate or under-reserved, amino acids will be used as substrates for gluconeogenesis, ketogenesis, and protein synthesis, leading to

post-exercise protein catabolism thereby increasing the concentration of amino acids in the blood (Schranner et al., 2020).

In this experiment, hypoxanthine (Hx) levels were found to be significantly elevated in athletes after fatigue from high-intensity intermittent exercise. Hypoxanthine is a naturally occurring purine derivative, which is both an intermediate metabolite in the purine metabolic pathway and an important myometabolite that is mainly distributed in muscle tissue (Finsterer, 2012). During exercise or muscle contraction, the continuous consumption of adenosine triphosphate (ATP) is accompanied by the production of intermediate metabolites such as adenosine monophosphate (AMP) and inosine monophosphate (IMP), which in turn form Hx (Yin et al., 2021). It has been reported that strenuous exercise increases adenine nucleotide metabolism (Sutton et al., 1980) and hypoxanthine concentration reaches its peak 10–20 min after high-intensity exercise (Sahlin et al., 1991). Other studies have suggested that degradation products such as inosine, hypoxanthine and xanthine increase in either mode of exercise, but that resistance exercise specifically leads to a sustained increase in nucleotide metabolites (e.g., xanthine) (Morville et al., 2020) has also been reported that exercise intensity and duration are key parameters in determining plasma Hx concentrations and purine nucleotide metabolism after exercise, and hypoxanthine can be considered a marker of anaerobic metabolism (Zieliński et al., 2015). Since serum hypoxanthine is directly related to the amount of intracellularly consumed ATP, the magnitude of the increase in blood Hx levels depends on the different exercise intensities and its value may be 2-10 times higher than at rest, so it is both a good biomarker of muscle fatigue and a sensitive metabolic indicator for evaluating the training level and training status of high-level athletes, and lower purine concentrations during competition indicate better adaptation to high-intensity exercise after training. The lower purine concentration during competition indicates better adaptation to high intensity exercise after training (Zieliński et al., 2015). Pyruvate is the end product of the glycolytic pathway of the body and is one of the important indicators of the degree of hypoxia in body tissues. This experiment also found that the elevation of pyruvate in long-distance runners after repeated high-intensity exercise training indicated that the body mainly used anaerobic enzymolysis of sugar to provide energy during exercise training, which is consistent with the study of Berton (Berton et al., 2017), whose literature reported a significant increase in serum pyruvate concentration after resistance training. Therefore, changes in pyruvate content can be used as an indicator of the body's energy metabolism mode before and after exercise (Enea et al., 2010). The increase in pyruvate and hypoxanthine concentrations following high-intensity interval exercise-induced fatigue suggests that the body's energy metabolism during exercise training is mainly dependent on the glycolytic energy supply system and the level of ATP turnover.

In this experiment, it was found that significant differential metabolites of sweat from athletes after high-intensity interval training could be used as a major biomarker reflecting the production of exercise-induced fatigue, and the metabolic pathways involved mainly in purine metabolism, tryptophan metabolism, alanine, aspartate and glutamate metabolism, and tyrosine metabolism. This is similar to related studies that reported results finding significant differences in differential metabolites in urine before and after marathon exercise mainly in metabolic pathways such as amino acid metabolism and riboflavin metabolism (Amelio et al., 2014; Pedley and Benkovic, 2017; Qin, 2021). In contrast, the purine metabolic pathway is mainly through the biosynthesis, degradation and interconversion of purines, which are essential for the structure of DNA and RNA, energy production and overall metabolism (Yin et al., 2018). The biological functions of the alanine, aspartate and glutamate metabolic pathways are the elimination of toxic ammonia and the replenishment of glucose in muscle through the interconversion of alanine to pyruvate and the transfer between skeletal muscle and the liver to meet the energy expenditure due to intense exercise, while alanine, aspartate and glutamate metabolism play a role in amino acid metabolism by transferring amino acids, which directly or indirectly affecting antibody and glutamine formation (Shao et al., 2017; Harshman et al., 2021). After fatigue from high-intensity intermittent exercise, these differential metabolites and differential metabolic pathways reflect changes in the cellular utilization of energy metabolic pathways and biomarkers of exercise-induced fatigue in the athlete's organism during exercise training.

5 Conclusion

The metabolic group of sweat before and after high-intensity intermittent exercise-induced fatigue was obvious, in which the upregulated differential metabolites were mainly hypoxanthine, pyruvate and several amino acids, while the downregulated differential metabolites were mainly amino acid derivatives, vitamin B6 and theophylline. Changes in sweat hypoxanthine concentration can be used as a good biomarker for the diagnosis of exercise-induced fatigue, while changes in sweat pyruvate content can be used as a discriminant indicator of the body's energy metabolism pattern before and after exercise. The metabolic pathways involved in the differential metabolites produced by athletes after high-intensity interval training mainly involve purine metabolism and amino acid metabolism. Sweat specimen collection from athletes is convenient and non-invasive, and sweat metabolomics analysis has good application for monitoring and evaluating the functional status of athletes.

Research limitations

Due to the regional variability, population variability, exercise specific variability and small sample size of this study, then a large-sample, multi-center, and multi-regional study is needed to verify the stability and reliability of this conclusion.

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 humans were approved by the Jimei University Academic and Ethical Review Council. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

SM: Writing–review and editing, Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing–original draft. JJ: Conceptualization, Data curation, Methodology, Software, Validation, Writing–review and editing, Writing–original draft. LY: Formal Analysis, Investigation, Methodology, Software, Validation, Writing–review and editing. ZS: Data curation, Investigation, Validation, Writing–original draft. ZJ: Investigation, Methodology, Software, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by Fujian Province Natural Science Fund of Grant No. 2019J01754, also it is supported by the cultivation grant (No. C520132) from Jimei University. The author highly recognizes Xiamen Meliomics Technology Co., Ltd.'s excellent technical support.

Conflict of interest

Authors ZS and ZJ were employed by Xiamen Meliomics Technology Co., LTD.

The remaining 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.

Publisher's note

References

Amelio, I., Cutruzzolá, F., Antonov, A., Agostini, M., and Melino, G. (2014). Serine and glycine metabolism in cancer. *Trends Biochem. Sci.* 39 (4), 191–198. doi:10.1016/j.tibs.2014.02.004

Berton, R., Conceição, M. S., Libardi, C. A., Canevarolo, R. R., Gáspari, A. F., Chacon-Mikahil, M. P. T., et al. (2017). Metabolic time-course response after resistance exercise: a metabolomics approach. *J. Sports Sci.* 35 (12), 1211–1218. doi:10.1080/02640414.2016.1218035

Cai, M., Wu, C., Jing, C., Shen, X., He, M., Wang, L., et al. (2022). Blood metabolomics analysis identifies differential serum metabolites in elite and sub-elite swimmers. *Front. Physiol.* 13, 858869. doi:10.3389/fphys.2022.858869

Delgado-Povedano, M. M., Calderón-Santiago, M., Luque de Castro, M. D., and Priego-Capote, F. (2018). Metabolomics analysis of human sweat collected after moderate exercise. *Talanta* 177, 47–65. doi:10.1016/j.talanta.2017.09.028

Enea, C., Seguin, F., Petitpas-Mulliez, J., Boildieu, N., Boisseau, N., Delpech, N., et al. (2010). (1)H NMR-based metabolomics approach for exploring urinary metabolome modifications after acute and chronic physical exercise. *Anal. Bioanal. Chem.* 396 (3), 1167–1176. doi:10.1007/s00216-009-3289-4

Finsterer, J. (2012). Biomarkers of peripheral muscle fatigue during exercise. *BMC Musculoskelet. Disord.* 13, 218–231. doi:10.1186/1471-2474-13-218

Harshman, S. W., Strayer, K. E., Davidson, C. N., Pitsch, R. L., Narayanan, L., Scott, A. M., et al. (2021). Rate normalization for sweat metabolomics biomarker discovery. *Talanta* 223 (1), 121797. doi:10.1016/j.talanta.2020.121797

Hooton, K., Han, W., and Li, L. (2016). Comprehensive and quantitative profiling of the human sweat submetabolome using high-performance chemical isotope labeling LC-MS. *Anal. Chem.* 88 (14), 7378–7386. doi:10.1021/acs.analchem.6b01930

Khoramipour, K., Sandbakk, Ø., Ammar Hassanzadeh, K., Gaeini, A. A., Wishart, D. S., and Chamari, K. (2021). Metabolomics in exercise and sports: a systematic Review. *Sports Med.* 52 (3), 547–583. doi:10.1007/s40279-021-01582-y

Manoel, F. A., Peserico, C. S., and Machado, F. A. (2022). Novel track field test to determine Vpeak, relationship with treadmill test and 10-km running performance in trained endurance runners. *PLoS One* 17 (1), e0260338. doi:10.1371/journal.pone.0260338

María Adeva-Andany, M., Pérez-Felpete, N., Fernández-Fernández, C., Donapetry-García, C., and Pazos-García, C. (2016). Liver glucose metabolism in humans. *Biosci. Rep.* 36 (6), 11–26. doi:10.1042/BSR20160385

Marinho, A. H., Sousa, F. A. D. B., Vilela, R. d.A. M. P., Balikian, P., Jr, de Souza Bento, E., de Mendonça Aquino, T., et al. (2021). The rating of perceived exertion is able to differentiate the post-matches metabolomic profile of elite U-20 soccer players. *Eur.J. Appl.Physiol* 122, 371–382. doi:10.1007/s00421-021-04838-7

Meeusen, R., Van Cutsem, J., and Roelands, B. (2021). Endurance exercise-induced and mental fatigue and the brain. *Exp. Physiol.* 106 (12), 2294–2298. doi:10.1113/EP088186

Morville, T., Sahl, R. E., Moritz, T., Helge, J. W., and Clemmensen, C. (2020). Plasma metabolome profiling of resistance exercise and endurance exercise in humans. *Cell Rep.* 33 (13), 108554. doi:10.1016/j.celrep.2020.108554

Pedley, A. M., and Benkovic, S. J. (2017). A new view into the regulation of purine metabolism: the purinosome. *Trends Biochem. Sci.* 42 (2), 141–154. doi:10.1016/j.tibs.2016.09.009

Qin, S. (2021). *Urine proteomics and metabolomics of male amateur runners before and after marathon exercise*. Shanghai, China: Shanghai Institute of Physical Education.

Sahlin, K., Ekberg, K., and Cizinsky, S. (1991). Changes in plasma hypoxanthine and free radical markers during exercise in man. *Acta Physiol. Scand.* 142 (2), 275–281. doi:10.1111/j.1748-1716.1991.tb09157.x

Sampson, D. L., Broadbent, J. A., Parker, A. W., and Upton, Z. (2014). Urinary biomarkers of physical activity: candidates and clinical utility. *Expert Rev. Proteomics* 11 (1), 91–106. doi:10.1586/14789450.2014.859527

Schranner, D., Kastenmüller, G., Schönfelder, M., Römisch-Margl, W., and Wackerhage, H. (2020). Metabolite concentration changes in humans after a bout of exercise: a systematic Review of exercise metabolomics studies. *Sports Med. Open* 6, 11–28. doi:10.1186/s40798-020-0238-4

Shao, C., Ren, Y., Wang, Z., Kang, C., Jiang, H., and Chi, A. (2017). Detection of urine metabolites in a rat model of chronic fatigue syndrome before and after exercise. *Biomed. Res. Int.* 2017, 8182020. doi:10.1155/2017/8182020

She, P., Zhou, Y., Zhang, Z., Griffin, K., Gowda, K., and Lynch, C. J. (2010). Disruption of BCAA metabolism in mice impairs exercise metabolism and endurance. *J. Appl. Physiology* 108 (4), 941–949. doi:10.1152/japplphysiol.01248.2009

Song-Gyu, Ra, Seiji, M., Ryota, H., Imai, T., and Miyakawa, S. (2014). Metabolomics of salivary fatigue markers in soccer players after consecutive games. *Appl. Physiology. Nutr. Metabolism* 39 (10), 1120–1126. doi:10.1139/apnm-2013-0546

Souza, S. L., Graça, G., and Oliva, A. (2018). Characterization of sweat induced with pilocarpine, physical exercise, and collected passively by metabolomic analysis. *Skin. Res. Technol.* 24 (2), 187–195. doi:10.1111/srt.12412

Stander, Z., Luies, L., Mienie, L. J., Keane, K. M., Howatson, G., Clifford, T., et al. (2018). The altered human serum metabolome induced by a marathon. *Metabolomics* 14 (11), 150–161. doi:10.1007/s11306-018-1447-4

Sutton, J. R., Toews, C. J., Ward, G. R., and Fox, I. H. (1980). Purine metabolism during strenuous muscular exercise in man. *Metabolism* 29 (3), 254–260. doi:10.1016/0026-0495(80)90067-0

Yin, C., Ma, Z., Li, F., Duan, C., Yuan, Y., Zhu, C., et al. (2021). Hypoxanthine induces muscular ATP depletion and fatigue via UCP2. *Front. Physiol.* 12, 647743. doi:10.3389/fphys.2021.647743

Yin, J., Ren, W., Huang, X., Deng, J., Li, T., and Yin, Y. (2018). Potential mechanisms connecting purine metabolism and cancer therapy. *Front. Immunol.* 9, 1697–2708. doi:10.3389/fimmu.2018.01697

Zhou, W., Zeng, G., Lyu, C., Kou, F., Zhang, S., and Wei, H. (2019). The effect of exhaustive exercise on plasma metabolic profiles of male and female rats. *J. Sports Sci. Med.* 18 (2), 253–263.

Zieliński, J., and Kusy, K. (2015). Hypoxanthine: a universal metabolic indicator of training status in competitive sports. *Exerc Sport Sci. Rev.* 43 (4), 214–221. doi:10.1249/JES.0000000000000055

frontiers | Frontiers in Physiology

TYPE Review
PUBLISHED 01 December 2023
DOI 10.3389/fphys.2023.1302769

OPEN ACCESS

EDITED BY
Cristina Vassalle,
Gabriele Monasterio Tuscany Foundation (CNR), Italy

REVIEWED BY
Zhiwen Luo,
Fudan University, China
Elen H. Miyabara,
University of São Paulo, Brazil

*CORRESPONDENCE
Lunhao Bai,
bailh@sj-hospital.org

RECEIVED 27 September 2023
ACCEPTED 21 November 2023
PUBLISHED 01 December 2023

CITATION
Jia S, Yu Z and Bai L (2023), Exerkines and osteoarthritis.
Front. Physiol. 14:1302769.
doi: 10.3389/fphys.2023.1302769

Exerkines and osteoarthritis

Shuangshuo Jia[1], Ziyao Yu[2] and Lunhao Bai[1]*

[1]Department of Orthopedic Surgery, Shengjing Hospital of China Medical University, Shenyang, China, [2]Imaging Department, Dalian Medical University, Dalian, China

Osteoarthritis (OA) is the most prevalent chronic joint disease, with physical exercise being a widely endorsed strategy in its management guidelines. Exerkines, defined as cytokines secreted in response to acute and chronic exercise, function through endocrine, paracrine, and/or autocrine pathways. Various tissue-specific exerkines, encompassing exercise-induced myokines (muscle), cardiokines (heart), and adipokines (adipose tissue), have been linked to exercise therapy in OA. Exerkines are derived from these kines, but unlike them, only kines regulated by exercise can be called exerkines. Some of these exerkines serve a therapeutic role in OA, such as irisin, metrnl, lactate, secreted frizzled-related protein (SFRP), neuregulin, and adiponectin. While others may exacerbate the condition, such as IL-6, IL-7, IL-15, IL-33, myostatin, fractalkine, follistatin-like 1 (FSTL1), visfatin, activin A, migration inhibitory factor (MIF), apelin and growth differentiation factor (GDF)-15. They exerts anti-/pro-apoptosis/pyroptosis/inflammation, chondrogenic differentiation and cell senescence effect in chondrocyte, synoviocyte and mesenchymal stem cell. The modulation of adipokine effects on diverse cell types within the intra-articular joint emerges as a promising avenue for future OA interventions. This paper reviews recent findings that underscore the significant role of tissue-specific exerkines in OA, delving into the underlying cellular and molecular mechanisms involved.

KEYWORDS

exerkine, osteoarthritis, exercise, myokine, cardiokine, adipokine

1 Introduction

Osteoarthritis (OA) is a prevalent and debilitating condition representing a steadily increasing and substantial health burden with deep implications for individuals, healthcare systems, and broader socioeconomic spheres. The cost of OA in the United States, Canada, United Kingdom, France and Australia has been estimated to account for between 1% and 2.5% of the gross national product of these countries (Hunter and Bierma-Zeinstra, 2019). Data from the Canadian cohort found that the average annual cost per OA patient was $12,200 (Hunter et al., 2014). Primary OA results from a combination of risk factors, such as genetics, dietary, estrogen, bone density, with increasing age and obesity being the most prominent (Yao et al., 2023). Globally, due to the aging global populations, the prevalence of OA has increased by 113.25%, from 247.51 million in 1990 to 527.81 million cases in 2019 (Long et al., 2022). Globally, an estimated 240 million individuals suffer from symptomatic mobility-restricted OA, with the knee being the most commonly affected joint and, thereby, the focal point of this review (Conaghan et al., 2019; Yao et al., 2023). Roughly 30% of individuals above the age of 45 exhibit radiographic indications of knee OA, half of whom experience symptoms associated with the disease (Katz et al., 2021). OA manifests through pathological alterations in cartilage, bone, synovium, tendons, muscles, and periarticular fat, engendering joint dysfunction, pain, stiffness, functional impairments, and the forfeiture of crucial activities (Abramoff and Caldera, 2020; Katz et al., 2021).

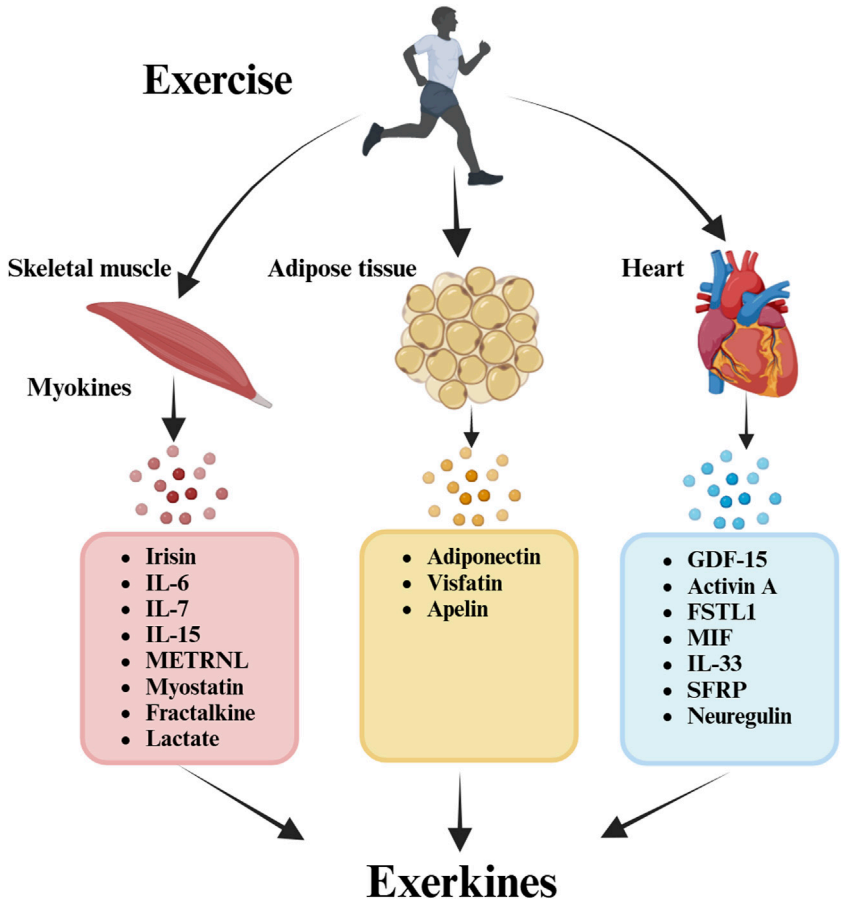


FIGURE 1
Exercise and exerkines. Exerkines involve various signaling entities secreted post-exercise, operating via endocrine, paracrine, and/or autocrine pathways. This analysis extends the exerkine classification to encompass exercise-associated humoral factors, including myokines (muscle), cardiokines (heart), and adipokines (white adipose tissue; WAT).

Current therapeutic approaches primarily encompass drug therapies such as non-steroidal anti-inflammatory drugs (NSAIDs) and joint replacement for advanced-stage disease; however, these strategies largely offer symptomatic relief (Latourte et al., 2020; Yue and Berman, 2022). In addition, NSAIDs have been shown to be associated with gastrointestinal, cardiovascular, renal, haematological and hepatic adverse events (AEs) (Holden et al., 2023). Joint replacement is only used for severe end-stage OA and imposes a heavy financial burden and pain on patients (Bandak et al., 2022). Recent clinical guidelines have increasingly advocated for non-surgical interventions such as physical exercise as frontline treatments (Hsu et al., 2023). Recognized as safe and efficacious, exercise therapy is gaining acceptance, with the World Health Organization recommending 150–300 min of moderate-intensity or 75–150 min of high-intensity physical activity weekly for early OA patients (Bennell et al., 2022; Chow et al., 2022). Over the past decade, a burgeoning body of evidence has affirmed the substantial impact of physical activity, notably moderate-intensity activity, on OA, corroborating the notion that "exercise is the real polypill," predicated on organ-induced peripheral factors (Torstensen et al., 2023). Generally, the therapeutic effects of exercise on OA are attributed to repeated exercise sessions, suggesting an association with cumulative acute responses to physical activity.

Central to this discussion is the concept of "exerkines," encompassing peptides, microRNAs, mRNAs, or other circulating RNA species released into the bloodstream in response to exercise (Lou et al., 2022; Robbins and Gerszten, 2023). This review delineates the potential function of exerkines in enhancing the benefits of exercise for OA patients. Exerkines involve various signaling entities secreted post-exercise, operating via endocrine, paracrine, and/or autocrine pathways (Chow et al., 2022). This analysis extends the exerkine classification to encompass exercise-associated humoral factors, including myokines (muscle), cardiokines (heart), and adipokines (white adipose tissue; WAT). Specially, only kines regulated by exercise can be called exerkines (Pillon et al., 2022; Hao et al., 2023). Recently, the pivotal role of physical exercise as a direct modulator enhancing general physiological aspects tied to OA has come to the fore, highlighting the exerkines' function in this realm. Understanding the mechanistic variability in exercise responses and their mediating roles in OA exercise therapy is paramount, necessitating further exploration of the involved processes and mechanisms.

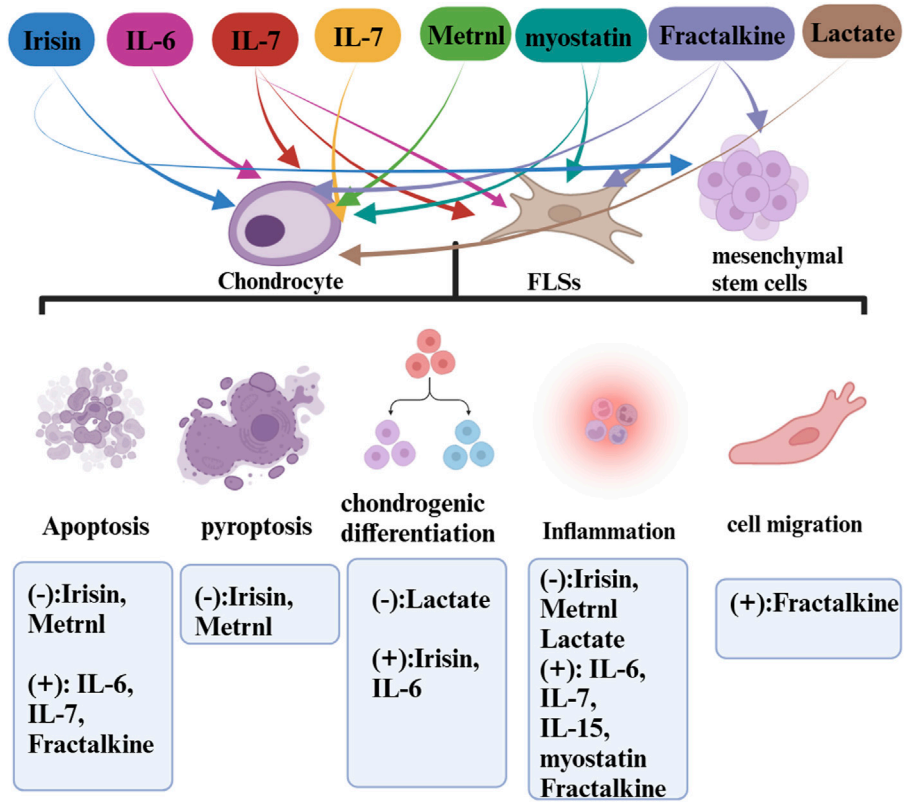


FIGURE 2
The roles of exerkines (myokines) in OA.

The novelty of this review compared to previous research is that we firstly reviewed the roles of exerkines in exercise therapy of OA. This review aims to encapsulate the current understanding of exerkines, underscoring their significance, delineating prevailing challenges (the specific molecular agents and mechanism remain unclear in the exercise therapy of OA), and envisioning prospective trajectories (exploring preclinical translational potentials, such as elucidating exerkine-associated effects and understanding individual physiological responses to different exercise interventions) in this burgeoning field.

2 Myokines

Skeletal muscle (SkM), the body's largest organ, is closely associated with physical activity (Pedersen and Febbraio, 2008a). Recently, SkM has been recognized as a secreting organ that produces and releases cytokines called "myokines" (Pedersen and Febbraio, 2008a). These myokines—cytokines or peptides generated by skeletal muscle cells—find their way into the circulation, influencing other cells, tissues, or organs through autocrine, paracrine, or endocrine effects (Kurdiova et al., 2014; Pedersen, 2019; Severinsen and Pedersen, 2020). Several myokines, including irisin, interleukin-6 (IL-6), interleukin-15 (IL-15), meteorin-like (METRNL), and β-aminoisobutyric acid (BAIBA), are consistently released by SkM in response to exercise, playing a pivotal role in mediating the beneficial impacts of physical activity (Benatti and Pedersen, 2015; Paris et al., 2020).

2.1 Irisin

In 2012, Bostrom et al. (2012) uncovered that physical exercise stimulates muscles to secrete irisin, a hormone-like polypeptide derived from the cleavage of the fibronectin type III domain-containing protein 5 (FNDC5). Comprising 112 amino acid residues, irisin possesses a molecular weight of roughly 12 kD. Subsequent research established that exercising augments the expression of peroxisome proliferator-activated receptor-γ (PPAR-γ) and its coactivator-1-α (PGC-1α) in muscles, facilitating the downstream production of FNDC5 and its proteolytic conversion into irisin (Kim et al., 2018; Maak et al., 2021; Bao et al., 2022).

Vadala et al. (2020) were the first to demonstrate that irisin could potentially rejuvenate osteoarthritic chondrocytes, encouraging their proliferation while curtailing catabolism by deactivating p38, Akt, JNK, and NFκB *in vitro*, thus suggesting a cross-signaling mechanism between muscle and cartilage. Further, Wang et al. (2020) associated FNDC5 signaling disruption during OA knee development with increased chondrocyte apoptosis, whereas irisin was found to inhibit defective autophagy and enhance inflammatory chondrocyte and extracellular matrix (ECM) anabolism survival via the UCP-1 and Sirt3 signaling pathways. Li et al. (2021) noted that irisin could attenuate OA progression by reducing cartilage degradation and mitigating inflammation.

Jia et al. (2022) revealed that moderate-intensity treadmill exercise elevated irisin levels, exerting beneficial therapeutic

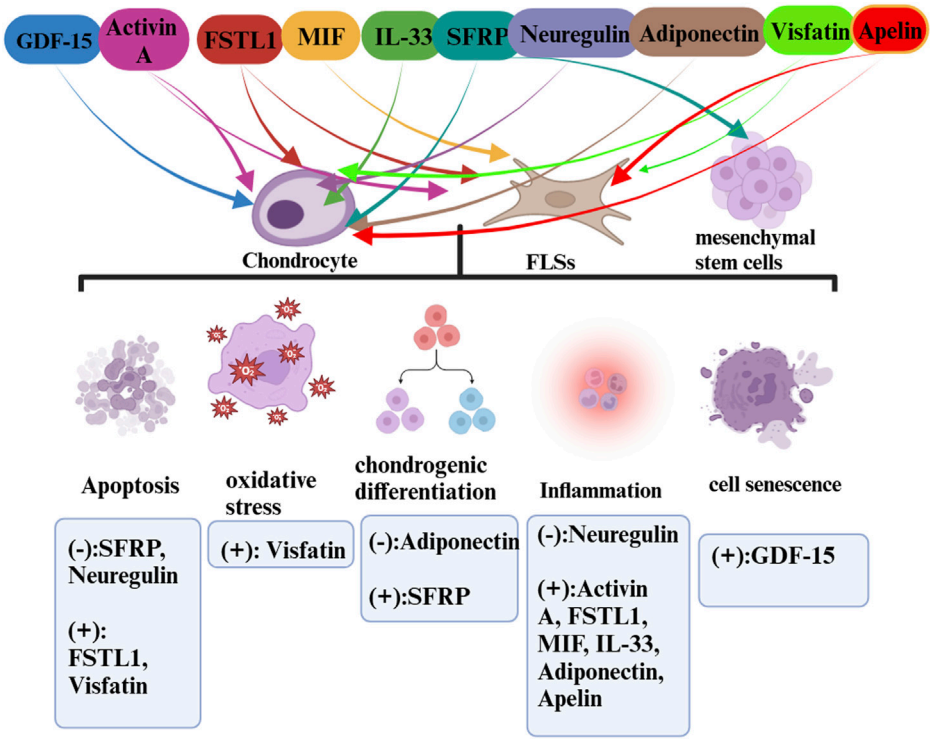


FIGURE 3
The roles of exerkines (cardiokines and adipokines) in OA.

effects on OA. These effects could be dampened using irisin-neutralizing antibodies. Moreover, irisin restored collagen II expression and reduced MMP-13 and ADAMTS-5 levels in IL-1β-induced OA chondrocytes by suppressing the PI3K/Akt/NF-κB signaling pathway and diminished pyroptosis in chondrocytes by inhibiting NLRP3/caspase-1 activity. The research concluded moderate mechanical stimulation could guard against chondrocyte pyroptosis through irisin-mediated PI3K/Akt/NF-κB pathway inhibition in osteoarthritis.

Further studies by He et al. (2020a) showed that irisin activated the ERK and p38 signaling pathways, with its anti-apoptotic effect reliant on ERK signaling, thus aiding in slowing OA progression by decreasing osteocyte apoptosis and enhancing subchondral bone microarchitecture. Additionally, irisin fostered chondrogenic differentiation in three-dimensional cultures of human articular chondrocytes (Posa et al., 2023). Cumulatively, these studies spotlight irisin's promising role in augmenting bone density, resisting cartilage degradation, and maintaining joint environmental homeostasis through exercise training.

2.2 Interleukin-6 (IL-6)

During physical activity, contracting skeletal muscles extensively release interleukin-6 (IL-6) into the circulatory system (Pedersen and Febbraio, 2008b). Being a pleiotropic cytokine, IL-6 exhibits increased concentration in both the serum and synovial fluid of OA patients, establishing a correlation with radiographic knee OA (Hunter and Jones, 2015; Jones and Jenkins, 2018). Notably, about 50% of individuals with osteoarthritis endure synovitis, a significant contributor to IL-6 production in the condition (D'Agostino et al., 2005; Nguyen et al., 2017).

IL-6 operates through two principal pathways: classic signaling, facilitated by membrane-anchored IL-6R (mIL-6R), and trans-signaling, involving a soluble form of IL-6R (sIL-6R), which broadens the array of IL-6 target cells primarily guiding pro-inflammatory events (Kelly et al., 2021; Rodríguez-Hernández et al., 2022). The function of IL-6 in OA, however, remains a subject of dispute. Numerous investigations have linked heightened IL-6 levels in circulation to the prediction of osteoarthritis and cartilage deterioration, steering the potentiation of inflammatory repercussions in affected joints and promoting the creation of enzymes that break down chondrocyte ECM (Rose-John, 2012; Bergmann et al., 2017).

Yang et al. (2017) reported that DNA hypomethylation and histone hyperacetylation were observed in the IL-6 promoter regions in OA synovial fluid. Suzuki et al. (2010) reported that IL-6 induces synovial cells to produce MMP-1, MMP-3, and MMP-13. Nasi et al. (2016) found that basic calcium phosphate (BCP) crystal deposition increases in OA joints, stimulating IL-6 synthesis by articular chondrocytes. IL-6 stimulates IL-6 production in an autocrine manner and crystal deposition by inducing the calcification genes Ank, Anx5, and Pit1. This will result in the maintenance of BCP crystal-induced IL-6 production. This vicious circle induces cartilage matrix-degrading enzymes (such as Mmp-3 and Mmp-13 and Adamts-4 and Adamts-5) in chondrocytes, and subsequent chondrodegradation occurs.

However, some studies also showed that IL-6 levels were elevated in joints with symptomatic cartilage defects or

TABLE 1 The roles of exerkines (myokines) in OA.

Exerkines	Origin	Effects in tissue	Immunomodulatory properties	Signaling pathway	Reference
Irisin (also known as FNDC5)	Myokine	Chondrocyte, mesenchymal stem cells	Positive correlation: anti-apoptosis; decreasing cartilage degradation and inhibiting inflammation and pyroptosis; chondrogenic differentiation	p38, Akt, JNK, NFκB, UCP-1, Sirt3,NLRP3, caspase-1, ERK	He et al., (2020a), Vadala et al., (2020), Wang et al., (2020), Li et al., (2021), Bao et al., (2022), Jia et al., (2022)
Interleukin-6	Myokine	Chondrocyte, FLSs	Positive correlation: promote the anabolism of chondrocytes; facilitate regeneration; production of anti-catabolic cytokines Negative correlation: increase synovitis; amplifies the inflammatory effects; DNA hypomethylation and histone hyperacetylation; produce MMPs; chondrodegradation	mIL-6R,sIL-6R, IL-6 trans-signaling	Silacci et al., (1998), Dagostino et al., (2005), Pedersen and Febbraio, (2008b), Andersson et al., (2010), Haugen et al., (2010), Suzuki et al., (2010), Rose-John, (2012), Tsuchida et al., (2012), Hunter and Jones, (2015), Nasi et al., (2016), Yang et al., (2017), Wiegertjes et al., (2020)
Interleukin-7	Myokine	Chondrocyte, FLSs	Negative correlation: cartilage destruction; increased in the SF of elderly with different degrees of OA	N/A	Pedersen, (2011), Steel et al., (2012), Crane et al., (2015), Stone et al., (2015)
Interleukin-15	Myokine	Chondrocyte	Negative correlation: recruitment or survival of CD8 lymphocytes; biomarker of disease severity; correlated with OA pain; increased protease and MMPs production	N/A	Rao et al., (2014), Jung et al., (2018), Warner et al., (2020), Gao et al., (2022), Lu et al., (2022)
Metrnl	Myokine	Chondrocyte, FLSs	Positive correlation: anti-inflammatory and antipyroptotic	PI3K/Akt/NFκB, NLRP3/caspase-1/ GSDMD	Altin and Schulze (2011), Dankbar et al. (2015), Kim et al. (2021), Omosule and Phillips (2021), Liu et al. (2022)
Myostatin (also known as GDF8)	Myokine	Synoviocytes, chondrocyte	Negative correlation: correlate with the severity of OA and inflammatory cytokines	N/A	Leonov et al. (2011), Catoire et al. (2014), Crabb et al. (2016), Wojdasiewicz et al. (2020)
Fractalkine (also known as CX3CL1)	Myokine	Mesenchymal stem cells, chondrocyte, FLSs	Negative correlation: bone resorption; increase apoptosis; mediates cellular adhesive and migratory functions; migration of OA FLSs	CX3CR1, c-Raf, MEK, ERK, and NF-κB	Borchers and Pieler (2010), Zhang et al., (2016b), Covington et al., (2016), Hou et al., (2017), Farah et al., (2022), Brooks et al., 2023
Lactate	Myokine	Chondrocyte	Positive correlation: promoting chondrocyte ECM synthesis and Col2α1 expression	N/A	Klein et al., (2021), Kim et al., (2022)

osteoarthritis compared to healthy joints (Tsuchida et al., 2012). In addition, elevated IL-6 levels appear to promote the anabolism of resident chondrocytes and appear to facilitate the formation of new cartilage during *in vitro* regeneration (Tsuchida et al., 2012). Soluble IL-6R also augmented the production of anti-catabolic TIMPs in chondrocytes (Silacci et al., 1998), which suggests the direct role of IL-6 in regulating chondrocyte function and cartilage metabolism. This dual role of IL-6 is incompletely understood and may be caused by differential effects of IL-6 classic vs. trans-signaling (Wiegertjes et al., 2020).

2.3 Interleukin-7 (IL-7)

IL-7, a 25 kDa secreted globular protein encoded by the IL7 gene, has been discerned in the culture medium of human muscle tube primary cultures derived from satellite cells, with its concentration escalating over the cultivation period (Haugen et al., 2010). This molecule, called an "exerkine," finds release into the bloodstream amid exercise (Andersson et al., 2010; Haugen et al., 2010).

Although it has been linked with osteoarthritis, the role of IL-7 in this context is mainly detrimental, contributing to the wreckage of cartilage in various joint disorders, including OA (Long et al., 2008; Zhang H. X. et al., 2016). Notably, IL-7 levels spike in the synovial fluid (SF) of older individuals with varying OA severities; however, it diminishes in cases of severe compartment 3 osteoarthritis, presumably owing to the substantial damage to cartilage cells enmeshed in the affected tissue (Long et al., 2008). Moreover, analysis in vervet monkeys indicated an elevated secretion of IL-7 in elderly meniscus samples with osteoarthritic changes compared to healthy counterparts, designating IL-7 as a marker rather than a therapeutic agent in OA management (Stone et al., 2015). The underlying consensus depicts IL-7 more as a marker for osteoarthritis rather than a therapeutic avenue leveraged through exercise.

2.4 Interleukin-15 (IL-15)

Interleukin-15 (IL-15), a glycoprotein comprising four intertwined α-helices and having a mass of 14–15 kDa (Steel et al., 2012), operates as an exercise-induced myokine, witnessing an uptick in its secretion from skeletal muscles post-exercise (Pedersen, 2011; Crane et al., 2015; Kurz et al., 2022). Early-stage knee OA patients have been observed to have elevated levels of IL-15 in the SF compared to those at the end stage (Scanzello et al., 2009).

TABLE 2 The roles of exerkines (cardiokines and adipokines) in OA.

Exerkines	Origin	Effects in tissue	Immunomodulatory properties	Signaling pathway	Reference
GDF-15	cardiokine	chondrocyte	Negative correlation: cellular senescence	MAPK14	Bennell et al. (2022)
Activin A	cardiokine	chondrocyte, FLSs	Positive correlation: anabolic factor in cartilage Negative correlation: correlate with inflammatory cytokines	mIL-6R, sIL-6R, IL-6 trans-signaling	Silacci et al. (1998), Dagostino et al. (2005), Pedersen and Febbraio (2008b), Andersson et al. (2010), Haugen et al. (2010), Suzuki et al. (2010), Rose-John (2012), Tsuchida et al. (2012), Hunter and Jones (2015), Nasi et al. (2016), Yang et al. (2017), Wiegertjes et al. (2020)
FSTL1	Cardiokine	Chondrocyte, FLSs	Negative correlation: along the interface of eroding bone and inflammatory synovial pannus; pro-inflammatory in OA; FLSs proliferation; promote chondrocytes apoptosis	NF-κB pathway, SAPK/JNK/Caspase3	Calandra and Roger (2003), Liu and Hu (2012), Moon et al. (2013), Chaly et al. (2015), Ni et al. (2015), Chang et al., (2019), Hu et al. (2019), Li et al. (2020), Xu et al. (2020)
MIF	Cardiokine	FLSs	Negative correlation: correlate with the severity of OA and related pain	N/A	Talabot-Ayer et al. (2012), Li et al. (2017), He et al. (2020b)
Interleukin-33	Cardiokine	Chondrocyte	Negative correlation: increase inflammation and cartilage degradation	N/A	Loughlin (2005), Thysen et al. (2015), Kim et al. (2017), Lee et al. (2019)
Secreted frizzled-related protein	Cardiokine	Chondrocyte, mesenchymal stem cells	Positive correlation: regulate chondrocyte differentiation and survival; modulate joint homeostasis; delaying the maturation of proliferative chondrocytes into hypertrophic chondrocytes	polymorphism in SFRP cis-acting regulatory elements,	Bouassida et al. (2010), Neumann et al. (2016), Macdonald et al. (2019), Martinez-Huenchullan et al. (2020), Yuan et al. (2023)
Neuregulin	Cardiokine	Chondrocyte	Positive correlation: inhibiting the inflammation, protecting against apoptosis of chondrocyte, and decreasing the degradation of extracellular matrix	MAPK, JNK	Challa et al. (2010)
Adiponectin	Adipokines	Chondrocyte	Positive correlation: increase proliferation of chondrocytes and the type II collagen and aggrecan; alleviates the calcification of OA chondrocytes; matrix remodelling	AMPK, mTOR	Chen et al. (2010), Seo et al. (2011), Cheleschi et al. (2018), Franco-Trepat et al. (2019b), Cheleschi et al. (2019), Law et al. (2020), Chang et al. (2021)
Visfatin	Adipokines	Chondrocyte, FLSs	Negative correlation: enhances intercellular adhesion molecule type 1 expression; facilitates the adhesion of monocytes; induce apoptosis and oxidative stress; enhanced VEGF expression and facilitates angiogenesis; damages the microtubule and microfilament networks	GSK3β, ICAM-1, VEGF	Hu et al. (2010), Hehir and Morrison (2012), Yang et al. (2019), Barbalho et al. (2020), Chang et al. (2020), Yang et al. (2020), Luo et al. (2021), Bolander et al. (2023)
Apelin	adipokines	chondrocyte, FLSs	Negative correlation: exacerbated osteoarthritis pathogenesis; promoted chondrocyte proliferation and induced expression of MMPs and IL-1β	PI3K, ERK	Hu et al. (2010), Hu et al. (2011), Chang et al. (2020), Luo et al. (2021)

IL-15 seemingly aids in the recruitment or sustenance of CD8 lymphocytes within the joints of OA patients, hinting at its critical role in OA's pathogenesis (Scanzello et al., 2009).

According to various studies, serum IL-15 (sIL-15) levels indicate the severity of early arthritis, and a surge in these levels is independently associated with heightened self-reported pain in knee OA sufferers (Gonzalez-Alvaro et al., 2011; Sun et al., 2013). Although identified as a target for pain management, IL-15 signaling may not significantly affect OA's structural progression. Still, it does facilitate increased protease production in cartilage explants *in vitro* (Warner et al., 2020). It is regarded as a central regulator of MMPs, which spearhead cartilage loss in OA (Warner et al., 2020). Hence, targeting IL-15 signaling pathways could emerge as a pivotal strategy in OA therapeutic interventions, a premise necessitating more profound research.

2.5 Meteorin-like (METRNL)

Meteorin-like, or METRNL, is a circulating factor induced in muscles after exercise (Rao et al., 2014; Jung et al., 2018). Several evidence lines suggest anti-inflammatory and insulin-sensitizing effects of this myokine (Gao et al., 2022; Lu et al., 2022). In OA, Bridgewood et al. (2019) reported that METRNL is detectable in the SF of OA, which suggested that METRNL may participate in OA pathogenesis. Metrnl has also been reported to be expressed in hypertrophic chondrocytes. These hypertrophic chondrocytes have been reported as an essential event in the pathogenesis of osteoarthritis (Husa et al., 2010; Gong et al., 2016). The study also found that SF-metrnl was negatively associated with SF-MMP-13 and portrayed metrnl as a possible player in putative compensatory responses in OA (Sobieh et al., 2021). In other studies,

they set an exercise protocol and discovered the correlation between the degree of OA and concentration of metrnl in the serum and synovial fluid. Moreover, based on the pathogenesis of OA, they found that exercise-induced metrnl could ameliorate OA through its anti-inflammatory and antipyroptotic effects, which are mediated by suppressing the PI3K/Akt/NFκB and NLRP3/caspase-1/GSDMD pathways (Liu et al., 2022).

2.6 Myostatin

Myostatin or growth differentiation factor 8 (GDF8) elevates with exercise. It exhibits a higher serum concentration in OA patients, correlating with disease severity (Kim et al., 2021; Omosule and Phillips, 2021). Pro-inflammatory cytokines such as TNF-α, IL-1, and IL-17 enhance myostatin expression in human synoviocytes. Moreover, chronic exposure to inflammatory cytokines leads to escalated myostatin expression in the synovium of hTNFtg mice (Dankbar et al., 2015). These observations propose a promising pathway for osteoarthritis treatment, wherein targeting myostatin could potentially reduce inflammation and joint destruction, paving the way for a new pharmacological approach in OA management.

2.7 Fractalkine

Fractalkine (FKN), also known as CX3CL1, is a novel membrane-bound myokine mainly expressed in contracting muscle (Altin and Schulze, 2011). Several studies show that aerobic exercise significantly elevates the fractalkine (Catoire et al., 2014; Crabb et al., 2016). Fractalkine is involved in various normal and pathological processes and is considered relevant to joint degeneration (Leonov et al., 2011; Wojdasiewicz et al., 2020). Fractalkine was significantly upregulated when chondrocytes underwent apoptosis, and blockage of the CX3CL1-CX3CR1 (CX3CL1 receptor) axis resulted in less bone resorption in OA (Guo et al., 2022). Fractalkine mediates cellular adhesive and migratory functions and is known to be expressed in mesenchymal stem cells destined to become chondrocytes (Feldman et al., 2013). While scant, available data from the literature confirm that CX3CL1 and its receptor are involved in OA. However, some opposite effects have been reported. Fractalkine has been detected in patients with osteoarthritis (OA) in SFs. The chemokine domain of FKN effectively induces the migration of OA fibroblasts (Klosowska et al., 2009). In addition, it was also reported that fractalkine activates c-Raf, MEK, ERK, and NF-κB on the MMP-3 promoter through CX3CR1, thus contributing to cartilage destruction during OA (Hou et al., 2017). Independent studies have paved the way for using CX3CL1 as a valuable marker for determining OA severity or monitoring treatment outcomes. However, assessing the occurrence and role of fractalkine and its receptor requires further, more detailed studies.

2.8 Lactate

Lactate is no longer considered a metabolic waste and a cause of muscle fatigue (Borchers and Pieler, 2010). Depending on the condition, such as during rest and exercise, following carbohydrate nutrition, injury, or pathology, lactate can serve as a myokine or exerkine with autocrine-, paracrine-, and endocrine-like functions that have essential basic and translational implications (Covington et al., 2016). Lactate is an exerkine produced during exercise in the integument and working muscles (Brooks et al., 2023). OA patient SF exhibited significantly increased levels of lactate secretion and aerobic glycolysis (Farah et al., 2022).

In OA, a concentration of 100 mM lactate with an exposure duration of 8 h is optimal for promoting chondrocyte ECM synthesis (Zhang X. et al., 2016), and pulsed lactate addition induced more Col2α1 expression (Zhang X. et al., 2016). On the other hand, the study also found that acidic pH caused by lactate exerts adverse effects on chondrocyte proliferation and ECM expression (Zhang X. et al., 2016). These observed differential biological effects of lactate on chondrocytes would have implications for the future design of polymeric cartilage scaffolds.

3 Cardiokines

The secretomes produced by the heart during exercise encompass a group of proteins that have been referred to as cardiokines. Recent findings reinforce the notion of the heart as a secretory organ that produces a variety of cardiac factors that can influence the function of various cell types. Cardiac factors may also participate in virial processes by acting on distal metabolic tissues and affecting systemic homeostasis. Cardiac factors have protective and harmful effects in osteoarthritis. In the pathological process, the imbalance produced by cardiac factors may lead to the result of disease.

3.1 Growth differentiation factor (GDF)-15

A part of the transforming growth factor-β superfamily, GDF-15 or macrophage-inhibitory cytokine 1 has been highlighted in recent studies for its increased circulation levels following exercise, both in mice and humans (Shimano et al., 2012; Klein et al., 2021; Wang et al., 2021). The cardiokine, besides exhibiting anti-inflammatory properties, is known to curtail oxidative stress, thereby signaling its potential as a therapeutic target in OA, a disease often preceded by inflammation and oxidation (Arnold et al., 2020; Kim et al., 2022; Zhang et al., 2022).

A recent study pinpointed GDF-15 as a central player inducing cellular senescence in OA-afflicted chondrocytes through MAPK14 activation, thereby suggesting its substantial role in advancing OA (Borchers and Pieler, 2010). Further research into GDF-15 could potentially unveil novel pathways to target in the treatment and management of OA.

3.2 Activin A

Activin A, a member of the transforming growth factor-β superfamily, shows a marked increase in response to acute exercise (Perakakis et al., 2018). Identified as an exercise-induced cardiokine, activin A has heightened concentrations in the SF of

osteoarthritis (OA) patients. Furthermore, its production by synoviocytes and chondrocytes is stimulated in culture by inflammatory cytokines, including IL-1, TGF-beta, IFN-gamma, and IL-8 (Pignolo et al., 2019; Watt et al., 2020). Displaying immunomodulatory functions in OA, activin A governs the downregulation of type II collagen synthesis in articular cartilage at bone maturity and its reactivation during attempted OA repair, suggesting its role as a potential anabolic factor in cartilage (Hermansson et al., 2004; Diller et al., 2019).

3.3 Follistatin-like 1 (FSTL1)

FSTL1 originates from cardiomyocytes, endothelial cells, and smooth muscle cells (Xi et al., 2021). Exercise can enhance FSTL1 transcription in skeletal muscle and augment circulating FSTL1 levels (Görgens et al., 2013). Positioned as a potential biomarker of OA, FSTL1 can indicate the severity of joint damage, exhibiting a high presence at the juncture of eroding bone and inflammatory synovial pannus (Thornton et al., 2002; Mobasheri, 2012). Studies reveal FSTL1's potential as a serum biomarker reflecting joint injury severity in OA patients (Wang et al., 2011; Elsadek et al., 2021). Specifically, it is a crucial pro-inflammatory factor in OA pathogenesis, fostering synoviocyte proliferation by activating the NF-κB pathway (Ni et al., 2015; Hu et al., 2019). Further research by Chaly et al. highlighted FSTL1's regulatory role in chondrocyte activities and its implication in promoting apoptosis via the SAPK/JNK/Caspase3 pathway (Chaly et al., 2015; Xu et al., 2020). Cumulatively, these insights advocate targeting FSTL1 in osteoarthritis treatment strategies (Li et al., 2020).

3.4 Migration inhibitory factor (MIF)

MIF, a macrophage cytokine, modulates inflammatory and immune responses (Calandra and Roger, 2003). Produced by cardiomyocytes in the heart, its secretion amplifies during exercise, earning it the classification of a cardiokine (Moon et al., 2013; Chang et al., 2019). Observations show elevated MIF levels in the serum and SF of knee OA patients, establishing a strong correlation with the disease's severity (Liu and Hu, 2012). Hence, MIF emerges as a potential new biomarker for evaluating knee OA risk and severity. Remarkably, MIF deletion can attenuate OA severity, with synovial fluid MIF levels independently associated with the severity of self-reported pain in OA patients (Zhang P. L. et al., 2016; Rowe et al., 2017).

3.5 Interleukin-33 (IL-33)

Primarily secreted by cardiac fibroblasts, IL-33 sees an uptick in expression and secretion in response to mechanical strain (Liew et al., 2016). Studies report a significant increase in IL-33 levels in OA chondrocytes, with double-stranded RNA promoting cartilage degeneration through the TLR3/IL-33 pathway (Li et al., 2017). These findings indicate that IL-33 chiefly originates from chondrocytes in an OA joint, not synovial fibroblasts, and plays a role in aggravating OA. Targeting IL-33 and enhancing IL-37 function may synergistically dampen inflammation, showcasing IL-33's potential as a therapeutic target in OA management (Talabot-Ayer et al., 2012; Li et al., 2017; He et al., 2020b).

3.6 Secreted frizzled-related protein (SFRP)

SFRPs are glycoproteins encompassing a frizzled-like cysteine-rich domain, functioning as an exercise-inducible cardiokine (van Loon et al., 2021). Among the SFRPs, SFRP4 mRNA is highly responsive to exercise, potentially making it a crucial biomarker for evaluating the benefits of long-term exercise (Kim et al., 2017; Lee et al., 2019).

SFRPs regulate chondrocyte differentiation and survival in cartilage, presenting a viable target for therapeutic interventions (Loughlin, 2005). In a study utilizing a destabilization of the medial meniscus model (DMM), SFRP1 modulates joint homeostasis differentially in distinct joint compartments (Thysen et al., 2015). Diminished expression of SFRP1 renders the articular cartilage susceptible to premature aging and osteoarthritis (OA) development (Pasold et al., 2013). Conversely, SFRP3 protects chondrocytes in healthy articular cartilage by delaying the transformation of proliferative chondrocytes into hypertrophic ones (Snelling et al., 2007). In sum, SFRPs, including SFRP-1, -3, and -4, hold differential roles in OA pathogenesis.

3.7 Neuregulin

In human cardiac endothelial cells, neuregulin expression elevates in response to exercise, with neuregulin-4 (NRG4) playing a notable role as a member of the EGF-like family of extracellular ligands. Activated through ErbB4 receptor tyrosine kinases on the cytomembrane, NRG4 hinders OA progression by reducing inflammation, safeguarding chondrocytes from apoptosis, and mitigating ECM degradation via the MAPK/JNK signaling pathway (Cai et al., 2016; Yuan et al., 2023).

4 Adipokines

Originating from white adipose tissue, adipokines encompass bioactive peptides or proteins, immune molecules, and inflammatory mediators, including adiponectin and leptin (Neumann et al., 2016; Macdonald et al., 2019). Their incremented synthesis through exercise makes them focal points in OA exercise therapy.

4.1 Adiponectin

Adiponectin, predominantly secreted by adipocytes, sees heightened levels during exercise, implicating it in OA pathophysiology (Simpson and Singh, 2008; Bouassida et al., 2010; Martinez-Huenchullan et al., 2020). While increased severity of OA correlates with a decline in adiponectin levels in plasma and SF, its expression remains higher in OA patients compared to healthy individuals, associating it with OA

prevalence (Honsawek and Chayanupatkul, 2010; Tang et al., 2018). Studies illustrate adiponectin's protective role against OA by promoting chondrocyte proliferation and upregulating type II collagen and aggrecan in chondrocytes (Challa et al., 2010). Moreover, it inhibits the mitigation of OA chondrocyte calcification by activating the AMPK-mTOR signaling pathway, fostering autophagy (Duan et al., 2020). Interestingly, a favorable adiponectin/leptin ratio correlates with reduced pain in severe knee OA cases, spotlighting adiponectin's potential as a surrogate biomarker for monitoring physical function in knee OA patients (Francin et al., 2014; Udomsinprasert et al., 2020).

4.2 Visfatin

Visfatin is a critical enzyme in essential cellular processes governed by NAD+, including aging, oxidative stress, and sirtuin signaling (Franco-Trepat et al., 2019a). This enzyme is notably increased following short-term moderate aerobic exercise, categorizing it as an exercise-induced adipokine (Seo et al., 2011; Plinta et al., 2012).

Visfatin has a pronounced role in the pathophysiology of osteoarthritis (OA), where it exhibits local effects on joints during the progression of the disease (Chen et al., 2010; Franco-Trepat et al., 2019b). Furthermore, visfatin elevates the expression of intercellular adhesion molecule type 1 (ICAM-1) in human OA synovial fibroblasts (OASFs), promoting the adherence of monocytes to OASFs and fostering angiogenesis through enhanced vascular endothelial growth factor (VEGF) expression (Law et al., 2020). It has also been associated with inducing apoptosis and oxidative stress in human OA chondrocytes (Cheleschi et al., 2018; Cheleschi et al., 2019).

From a biomechanical standpoint, visfatin disrupts microtubule and microfilament networks, influencing intracellular mechanics by decreasing intracellular elasticity and viscosity through glycogen synthase kinase 3β (GSK3β) inactivation (Chang et al., 2021). In summary, visfatin functions as a central catabolic agent in OA pathogenesis, mediating the deterioration of osteoarthritic cartilage (Yang et al., 2015).

4.3 Apelin

Apelin is a peptide ranging from 13 to 36 amino acids in length, whose biological functions are mediated through its specific receptor, APJ, a G-protein-coupled receptor with seven transmembrane domains (Vinel et al., 2018). Since its identification, apelin has been classified both as a myokine and an adipokine, and its levels are known to increase in response to exercise, categorizing it as an exerkine as well (Hehir and Morrison, 2012; Lee et al., 2022)].

In the context of OA, apelin is found in the SF, with its concentrations correlating positively with OA severity (Hu et al., 2011). This peptide aggravates OA pathogenesis through the apelin/APJ system, promoting chondrocyte proliferation and increasing the expression of catabolic factors such as MMP-1, -3, -9, and IL-1β. Moreover, it diminishes collagen II synthesis, highlighting its role as a catabolic factor in OA progression (Hu et al., 2010; Luo et al., 2021).

Research has indicated that reducing apelin expression can alleviate OA cartilage severity, presenting a potential therapeutic avenue (Chang et al., 2020). Apelin also augments IL-1β expression by activating the PI3K and ERK pathways in OASFs, underlining its contributive role in OA onset and/or progression (Chang et al., 2020). In light of these findings, apelin emerges as a significant factor in understanding the intricate pathophysiology of OA, offering a new perspective in OA research.

5 Discussion

Exercise is a pivotal facilitator of health benefits, fostering essential communication and coordination between various systems and organs [169]. While it is widely acknowledged that exercise is beneficial in mitigating the symptoms of osteoarthritis (OA), the underlying mechanisms and the extent of these benefits remain partially understood. The "exercise is medicine" paradigm stands to gain considerably by incorporating insights derived from exerkine research, thus opening new avenues in public health discourse (Figures 1–3).

OA is a heterogeneous and complicated disease which is characterized by articular cartilage damage, synovitis, subchondral bone remodeling and osteophyte formation (Holden et al., 2023). The susceptibility factors including systemic factors (genetics, aging, sex, race, physical labor, obesity, and hypertension) and local factors (abnormal joint strength lines, poor muscle strength, high-intensity exercise, and joint injury history) (Yao et al., 2023). The pathophysiological processes of OA involve multiple tissues and organs, and studying the communication between tissues is critical for exercise treatment of OA. In recent years, the skeletal muscle, heart, adipose tissue are considered secretory organs with endocrine functions that can produce and secrete exercise-related molecules which participate in exercise therapy of OA (Barbalho et al., 2020). Much of the existing research in this area has honed in on skeletal muscle as the principal source of exerkines. However, contemporary efforts are extending this focus to encompass other potential sources, including the heart and adipose tissues, to advance our understanding of how exercise aids in achieving health restoration and maintenance. Essentially, different bouts of exercise modulate the release of a variety of kines, namely, myokines, cardiokines, and adipokines, which were the focus of this review.

In detailing the influence of exercise-induced upregulation, we have excluded cytokines that are downregulated through an exercise from our discussion as they do not qualify as exerkines. Our analysis spans a range of myokines such as irisin, IL-6, IL-7, IL-15, METRNL, myostatin, fractalkine, and lactate, cardiokines like GDF-15, Activin A, FSTL1, MIF, IL-33, SFRP, and neuregulin, and adipokines such as adiponectin, visfatin, and apelin. Due to a scarcity of data, our discussion does not encompass the more recently identified exerkines and their potential roles in OA diseases. This review endeavors to bridge the gap between these disparate areas of study, presenting a consolidated view that hints at a larger, underlying theory. Firstly, exercise unleashes a complex network of endocrine interactions in which exerkines, released in response to exercise, interplay through inter-organ crosstalk and physiologic changes of OA. Secondly, the exerkines are released into the synovial

fluid (SF) through the blood circulation. SF is formed by the serum in the articular capsule and released into the articular cavity, where it contributes to the unique functional properties of articular surfaces, provides nutrients to the cartilage, constitutes the microenvironment of articular cells, and modulates cells activity (Bolander et al., 2023). Thirdly, the exerkines act on FLSs, chondrocytes, mesenchymal stem cells and interfere with cell biological processes such as apoptosis, pyroptosis, inflammation, chondro differentiation through signaling pathway transduction to alleviate OA.

While the health benefits of exerkines are well acknowledged, it is also apparent that not all factors positively influence OA during exercise. Despite being upregulated through exercise, some factors can have adverse effects, a phenomenon possibly influenced by the intensity and frequency of exercise and the origin of the exerkines (as illustrated in Tables 1, 2). This discrepancy stems from the multifaceted impact of exercise on knee OA, involving elements such as mechanical stimulation and exerkine release, among others. We previously demonstrated that exercise could affect cartilage, which had a dual effect on osteoarthritis (Yang et al., 2019; Yang et al., 2020; Jia et al., 2022). Adaptive intensity exercise can reduce the sensitivity of chondrocytes to inflammation. However, excessive exercise leads to progressive damage, inhibits matrix synthesis, and stimulates the production of matrix degrading enzymes. Secondly, exercise can also affect articular cartilage by altering the surrounding microenvironment, such as exerkines (Yang et al., 2019; Yang et al., 2020; Jia et al., 2022; Liu et al., 2022). Research shows that normal articular cartilage and chondrocytes do not reap therapeutic benefits from mechanical stimulation alone; overstimulation can lead to damage [32, 170]. In circumstances where high-intensity exercises apply excessive mechanical stress to the articular cartilage, the detrimental effects can outweigh the potential benefits of exerkines. Even some exerkines have potential adverse effects in OA therapy as described in this review. Therefore, maintaining a harmonious balance between exercise and exerkines is critical.

Future research endeavors must focus on exploring preclinical translational potentials, such as elucidating the clinical implications of exerkine-associated effects and understanding individual physiological responses to different exercise interventions. Given that the current body of exerkine research primarily consists of independent studies, there is a pressing need for research initiatives based on larger sample sizes and comprehensive panels to assess the cumulative impact of all exerkines, both at the joint level and in circulation. Finally, this review identifies gaps that remain in the field of exercise physiology science and opportunities that exist to translate biologic insights into osteoarthritis improvement.

Author contributions

SJ: Conceptualization, Formal Analysis, Investigation, Resources, Validation, Visualization, Writing–original draft, Writing–review and editing. ZY: Writing–review and editing. LB: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing–original draft, Writing–review and editing.

Funding

The authors declare financial support was received for the research, authorship, and/or publication of this article. This study was funded by the National Natural Science Foundation of China (82102613 and 82172479), the Fundamental Research Project of Liaoning Provincial Department of Education (LJKQZ2021028) and the Fundamental Research Project of plan to revitalize talents in Liaoning (XLYC2002029).

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.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abramoff, B., and Caldera, F. E. (2020). Osteoarthritis: pathology, diagnosis, and treatment options. *Med. Clin. North Am.* 104 (2), 293–311. doi:10.1016/j.mcna.2019.10.007

Altin, S. E., and Schulze, P. C. (2011). Fractalkine: a novel cardiac chemokine? *Cardiovasc Res.* 92 (3), 361–362. doi:10.1093/cvr/cvr272

Andersson, H., Bøhn, S. K., Raastad, T., Paulsen, G., Blomhoff, R., and Kadi, F. (2010). Differences in the inflammatory plasma cytokine response following two elite female soccer games separated by a 72-h recovery. *Scand. J. Med. Sci. Sports* 20 (5), 740–747. doi:10.1111/j.1600-0838.2009.00989.x

Arnold, N., Rehm, M., Büchele, G., Peter, R. S., Brenner, R. E., Günther, K. P., et al. (2020). Growth differentiation factor-15 as a potent predictor of long-term mortality among subjects with osteoarthritis. *J. Clin. Med.* 9 (10), 3107. doi:10.3390/jcm9103107

Bandak, E., Christensen, R., Overgaard, A., Kristensen, L. E., Ellegaard, K., Guldberg-Møller, J., et al. (2022). Exercise and education versus saline injections for knee osteoarthritis: a randomised controlled equivalence trial. *Ann. Rheum. Dis.* 81 (4), 537–543. doi:10.1136/annrheumdis-2021-221129

Bao, J. F., She, Q. Y., Hu, P. P., Jia, N., and Li, A. (2022). Irisin, a fascinating field in our times. *Trends Endocrinol. Metab.* 33 (9), 601–613. doi:10.1016/j.tem.2022.06.003

Barbalho, S. M., Flato, U. A. P., Tofano, R. J., Goulart, R. A., Guiguer, E. L., Detregiachi, C. R. P., et al. (2020). Physical exercise and myokines: relationships with sarcopenia and cardiovascular complications. *Int. J. Mol. Sci.* 21 (10), 3607. doi:10.3390/ijms21103607

Benatti, F. B., and Pedersen, B. K. (2015). Exercise as an anti-inflammatory therapy for rheumatic diseases-myokine regulation. *Nat. Rev. Rheumatol.* 11 (2), 86–97. doi:10.1038/nrrheum.2014.193

Bennell, K. L., Lawford, B. J., Keating, C., Brown, C., Kasza, J., Mackenzie, D., et al. (2022). Comparing video-based, telehealth-delivered exercise and weight loss programs with online education on outcomes of knee osteoarthritis: a randomized trial. *Ann. Intern Med.* 175 (2), 198–209. doi:10.7326/m21-2388

Bergmann, J., Müller, M., Baumann, N., Reichert, M., Heneweer, C., Bolik, J., et al. (2017). IL-6 trans-signaling is essential for the development of hepatocellular carcinoma in mice. *Hepatology* 65 (1), 89–103. doi:10.1002/hep.28874

Bolander, J., Moviglia Brandolina, M. T., Poehling, G., Jochl, O., Parsons, E., Vaughan, W., et al. (2023). The synovial environment steers cartilage deterioration and regeneration. *Sci. Adv.* 9 (16), eade4645. doi:10.1126/sciadv.ade4645

Borchers, A., and Pieler, T. (2010). Programming pluripotent precursor cells derived from Xenopus embryos to generate specific tissues and organs. *Genes (Basel)* 1 (3), 413–426. doi:10.3390/genes1030413

Bostrom, P., Wu, J., Jedrychowski, M. P., Korde, A., Ye, L., Lo, J. C., et al. (2012). A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 481 (7382), 463–468. doi:10.1038/nature10777

Bouassida, A., Chamari, K., Zaouali, M., Feki, Y., Zbidi, A., and Tabka, Z. (2010). Review on leptin and adiponectin responses and adaptations to acute and chronic exercise. *Br. J. Sports Med.* 44 (9), 620–630. doi:10.1136/bjsm.2008.046151

Bridgewood, C., Russell, T., Weedon, H., Baboolal, T., Watad, A., Sharif, K., et al. (2019). The novel cytokine Metrnl/IL-41 is elevated in Psoriatic Arthritis synovium and inducible from both entheseal and synovial fibroblasts. *Clin. Immunol.* 208, 108253. doi:10.1016/j.clim.2019.108253

Brooks, G. A., Osmond, A. D., Arevalo, J. A., Duong, J. J., Curl, C. C., Moreno-Santillan, D. D., et al. (2023). Lactate as a myokine and exerkine: drivers and signals of physiology and metabolism. *J. Appl. Physiol.* 134 (3), 529–548. doi:10.1152/japplphysiol.00497.2022

Cai, M. X., Shi, X. C., Chen, T., Tan, Z. N., Lin, Q. Q., Du, S. J., et al. (2016). Exercise training activates neuregulin 1/ErbB signaling and promotes cardiac repair in a rat myocardial infarction model. *Life Sci.* 149, 1–9. doi:10.1016/j.lfs.2016.02.055

Calandra, T., and Roger, T. (2003). Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat. Rev. Immunol.* 3 (10), 791–800. doi:10.1038/nri1200

Catoire, M., Mensink, M., Kalkhoven, E., Schrauwen, P., and Kersten, S. (2014). Identification of human exercise-induced myokines using secretome analysis. *Physiol. Genomics* 46 (7), 256–267. doi:10.1152/physiolgenomics.00174.2013

Challa, T. D., Rais, Y., and Ornan, E. M. (2010). Effect of adiponectin on ATDC5 proliferation, differentiation and signaling pathways. *Mol. Cell Endocrinol.* 323 (2), 282–291. doi:10.1016/j.mce.2010.03.025

Chaly, Y., Blair, H. C., Smith, S. M., Bushnell, D. S., Marinov, A. D., Campfield, B. T., et al. (2015). Follistatin-like protein 1 regulates chondrocyte proliferation and chondrogenic differentiation of mesenchymal stem cells. *Ann. Rheum. Dis.* 74 (7), 1467–1473. doi:10.1136/annrheumdis-2013-204822

Chang, M. C., Park, C. R., Rhie, S. H., Shim, W. H., and Kim, D. Y. (2019). Early treadmill exercise increases macrophage migration inhibitory factor expression after cerebral ischemia/reperfusion. *Neural Regen. Res.* 14 (7), 1230–1236. doi:10.4103/1673-5374.251330

Chang, S. F., Huang, K. C., Lee, K. H., Chiang, Y. C., Lee, W. R., Hsieh, R. Z., et al. (2021). Effects of visfatin on intracellular mechanics and catabolism in human primary chondrocytes through glycogen synthase kinase 3β inactivation. *Int. J. Mol. Sci.* 22 (15), 8107. doi:10.3390/ijms22158107

Chang, T. K., Wang, Y. H., Kuo, S. J., Wang, S. W., Tsai, C. H., Fong, Y. C., et al. (2020). Apelin enhances IL-1β expression in human synovial fibroblasts by inhibiting miR-144-3p through the PI3K and ERK pathways. *Aging* 12 (10), 9224–9239. doi:10.18632/aging.103195

Cheleschi, S., Giordano, N., Volpi, N., Tenti, S., Gallo, I., Di Meglio, M., et al. (2018). A complex relationship between visfatin and resistin and microRNA: an *in vitro* study on human chondrocyte cultures. *Int. J. Mol. Sci.* 19 (12), 3909. doi:10.3390/ijms19123909

Cheleschi, S., Tenti, S., Mondanelli, N., Corallo, C., Barbarino, M., Giannotti, S., et al. (2019). MicroRNA-34a and MicroRNA-181a mediate visfatin-induced apoptosis and oxidative stress via NF-κB pathway in human osteoarthritic chondrocytes. *Cells* 8 (8), 874. doi:10.3390/cells8080874

Chen, W. P., Bao, J. P., Feng, J., Hu, P. F., Shi, Z. L., and Wu, L. D. (2010). Increased serum concentrations of visfatin and its production by different joint tissues in patients with osteoarthritis. *Clin. Chem. Lab. Med.* 48 (8), 1141–1145. doi:10.1515/cclm.2010.230

Chow, L. S., Gerszten, R. E., Taylor, J. M., Pedersen, B. K., van Praag, H., Trappe, S., et al. (2022). Exerkines in health, resilience and disease. *Nat. Rev. Endocrinol.* 18 (5), 273–289. doi:10.1038/s41574-022-00641-2

Conaghan, P. G., Cook, A. D., Hamilton, J. A., and Tak, P. P. (2019). Therapeutic options for targeting inflammatory osteoarthritis pain. *Nat. Rev. Rheumatol.* 15 (6), 355–363. doi:10.1038/s41584-019-0221-y

Covington, J. D., Tam, C. S., Bajpeyi, S., Galgani, J. E., Noland, R. C., Smith, S. R., et al. (2016). Myokine expression in muscle and myotubes in response to exercise stimulation. *Med. Sci. Sports Exerc* 48 (3), 384–390. doi:10.1249/mss.0000000000000787

Crabb, E. B., Franco, R. L., Caslin, H. L., Blanks, A. M., Bowen, M. K., and Acevedo, E. O. (2016). The effect of acute physical and mental stress on soluble cellular adhesion molecule concentration. *Life Sci.* 157, 91–96. doi:10.1016/j.lfs.2016.05.042

Crane, J. D., MacNeil, L. G., Lally, J. S., Ford, R. J., Bujak, A. L., Brar, I. K., et al. (2015). Exercise-stimulated interleukin-15 is controlled by AMPK and regulates skin metabolism and aging. *Aging Cell* 14 (4), 625–634. doi:10.1111/acel.12341

Dagostino, M. A., Conaghan, P., Le Bars, M., Baron, G., Grassi, W., Martin-Mola, E., et al. (2005). EULAR report on the use of ultrasonography in painful knee osteoarthritis. Part 1: prevalence of inflammation in osteoarthritis. *Ann. Rheum. Dis.* 64 (12), 1703–1709. doi:10.1136/ard.2005.037994

Dankbar, B., Fennen, M., Brunert, D., Hayer, S., Frank, S., Wehmeyer, C., et al. (2015). Myostatin is a direct regulator of osteoclast differentiation and its inhibition reduces inflammatory joint destruction in mice. *Nat. Med.* 21 (9), 1085–1090. doi:10.1038/nm.3917

Diller, M., Frommer, K., Dankbar, B., Tarner, I., Hülser, M. L., Tsiklauri, L., et al. (2019). The activin-follistatin anti-inflammatory cycle is deregulated in synovial fibroblasts. *Arthritis Res. Ther.* 21 (1), 144. doi:10.1186/s13075-019-1926-7

Duan, Z. X., Tu, C., Liu, Q., Li, S. Q., Li, Y. H., Xie, P., et al. (2020). Adiponectin receptor agonist AdipoRon attenuates calcification of osteoarthritis chondrocytes by promoting autophagy. *J. Cell Biochem.* 121 (5-6), 3333–3344. doi:10.1002/jcb.29605

Elsadek, B. E. M., Abdelghany, A. A., Abd El-Aziz, M. A., Madkor, H. R., Abd Elrady Ahmed, A., Abd-Elghaffar, S. K., et al. (2021). Validation of the diagnostic and prognostic values of ADAMTS5 and FSTL1 in osteoarthritis rat model. *Cartilage* 13 (2_Suppl. l), 1263s–1273s. doi:10.1177/1947603519852405

Farah, H., Wijesinghe, S. N., Nicholson, T., Alnajjar, F., Certo, M., Alghamdi, A., et al. (2022). Differential metabotypes in synovial fibroblasts and synovial fluid in hip osteoarthritis patients support inflammatory responses. *Int. J. Mol. Sci.* 23 (6), 3266. doi:10.3390/ijms23063266

Feldman, G. J., Parvizi, J., Levenstien, M., Scott, K., Erickson, J. A., Fortina, P., et al. (2013). Developmental dysplasia of the hip: linkage mapping and whole exome sequencing identify a shared variant in CX3CR1 in all affected members of a large multigeneration family. *J. Bone Min. Res.* 28 (12), 2540–2549. doi:10.1002/jbmr.1999

Francin, P. J., Abot, A., Guillaume, C., Moulin, D., Bianchi, A., Gegout-Pottie, P., et al. (2014). Association between adiponectin and cartilage degradation in human osteoarthritis. *Osteoarthr. Cartil.* 22 (3), 519–526. doi:10.1016/j.joca.2014.01.002

Franco-Trepat, E., Alonso-Perez, A., Guillan-Fresco, M., Jorge-Mora, A., Gualillo, O., Gomez-Reino, J. J., et al. (2019a). Visfatin as a therapeutic target for rheumatoid arthritis. *Expert Opin. Ther. Targets* 23 (7), 607–618. doi:10.1080/14728222.2019.1617274

Franco-Trepat, E., Guillan-Fresco, M., Alonso-Perez, A., Jorge-Mora, A., Francisco, V., Gualillo, O., et al. (2019b). Visfatin connection: present and future in osteoarthritis and osteoporosis. *J. Clin. Med.* 8 (8), 1178. doi:10.3390/jcm8081178

Gao, X., Leung, T. F., Wong, G. W., Ko, W. H., Cai, M., He, E. J., et al. (2022). Meteorin-β/Meteorin like/IL-41 attenuates airway inflammation in house dust mite-induced allergic asthma. *Cell Mol. Immunol.* 19 (2), 245–259. doi:10.1038/s41423-021-00803-8

Gong, W., Liu, Y., Wu, Z., Wang, S., Qiu, G., and Lin, S. (2016). Meteorin-like shows unique expression pattern in bone and its overexpression inhibits osteoblast differentiation. *PLoS One* 11 (10), e0164446. doi:10.1371/journal.pone.0164446

Gonzalez-Alvaro, I., Ortiz, A. M., Alvaro-Gracia, J. M., Castaneda, S., Diaz-Sanchez, B., Carvajal, I., et al. (2011). Interleukin 15 levels in serum may predict a severe disease course in patients with early arthritis. *PLoS One* 6 (12), e29492. doi:10.1371/journal.pone.0029492

Görgens, S. W., Raschke, S., Holven, K. B., Jensen, J., Eckardt, K., and Eckel, J. (2013). Regulation of follistatin-like protein 1 expression and secretion in primary human skeletal muscle cells. *Arch. Physiol. Biochem.* 119 (2), 75–80. doi:10.3109/13813455.2013.768270

Guo, Y. N., Cui, S. J., Tian, Y. J., Zhao, N. R., Zhang, Y. D., Gan, Y. H., et al. (2022). Chondrocyte apoptosis in temporomandibular joint osteoarthritis promotes bone resorption by enhancing chemotaxis of osteoclast precursors. *Osteoarthr. Cartil.* 30 (8), 1140–1153. doi:10.1016/j.joca.2022.04.002

Hao, Z., Liu, K., Zhou, L., and Chen, P. (2023). Precious but convenient means of prevention and treatment: physiological molecular mechanisms of interaction between exercise and motor factors and Alzheimer's disease. *Front. Physiol.* 14, 1193031. doi:10.3389/fphys.2023.1193031

Haugen, F., Norheim, F., Lian, H., Wensaas, A. J., Dueland, S., Berg, O., et al. (2010). IL-7 is expressed and secreted by human skeletal muscle cells. *Am. J. Physiol. Cell Physiol.* 298 (4), C807–C816. doi:10.1152/ajpcell.00094.2009

He, Z., Li, H., Han, X., Zhou, F., Du, J., Yang, Y., et al. (2020a). Irisin inhibits osteocyte apoptosis by activating the Erk signaling pathway *in vitro* and attenuates ALCT-induced osteoarthritis in mice. *Bone* 141, 115573. doi:10.1016/j.bone.2020.115573

He, Z., Song, Y., Yi, Y., Qiu, F., Wang, J., Li, J., et al. (2020b). Blockade of IL-33 signalling attenuates osteoarthritis. *Clin. Transl. Immunol.* 9 (10), e1185. doi:10.1002/cti2.1187

Hehir, M. P., and Morrison, J. J. (2012). The adipokine apelin and human uterine contractility. *Am. J. Obstet. Gynecol.* 206 (4), 359.e351–e5. doi:10.1016/j.ajog.2012.01.032

Hermansson, M., Sawaji, Y., Bolton, M., Alexander, S., Wallace, A., Begum, S., et al. (2004). Proteomic analysis of articular cartilage shows increased type II collagen synthesis in osteoarthritis and expression of inhibin betaA (activin A), a regulatory molecule for chondrocytes. *J. Biol. Chem.* 279 (42), 43514–43521. doi:10.1074/jbc.M407041200

Holden, M. A., Nicolson, P. J. A., Thomas, M. J., Corp, N., Hinman, R. S., and Bennell, K. L. (2023). Osteoarthritis year in review 2022: rehabilitation. *Osteoarthr. Cartil.* 31 (2), 177–186. doi:10.1016/j.joca.2022.10.004

Honsawek, S., and Chayanupatkul, M. (2010). Correlation of plasma and synovial fluid adiponectin with knee osteoarthritis severity. *Arch. Med. Res.* 41 (8), 593–598. doi:10.1016/j.arcmed.2010.11.007

Hou, S. M., Hou, C. H., and Liu, J. F. (2017). CX3CL1 promotes MMP-3 production via the CX3CR1, c-Raf, MEK, ERK, and NF-κB signaling pathway in osteoarthritis synovial fibroblasts. *Arthritis Res. Ther.* 19 (1), 282. doi:10.1186/s13075-017-1487-6

Hsu, C. H., Huang, H. T., and Hsu, N. C. (2023). Diet and exercise and knee pain in patients with osteoarthritis and overweight or obesity. *Jama* 329 (15), 1317. doi:10.1001/jama.2023.2539

Hu, P. F., Chen, W. P., Tang, J. L., Bao, J. P., and Wu, L. D. (2010). Apelin plays a catabolic role on articular cartilage: *in vivo* and *in vitro* studies. *Int. J. Mol. Med.* 26 (3), 357–363. doi:10.3892/ijmm_00000473

Hu, P. F., Ma, C. Y., Sun, F. F., Chen, W. P., and Wu, L. D. (2019). Follistatin-like protein 1 (FSTL1) promotes chondrocyte expression of matrix metalloproteinase and inflammatory factors via the NF-κB pathway. *J. Cell Mol. Med.* 23 (3), 2230–2237. doi:10.1111/jcmm.14155

Hu, P. F., Tang, J. L., Chen, W. P., Bao, J. P., and Wu, L. D. (2011). Increased apelin serum levels and expression in human chondrocytes in osteoarthritic patients. *Int. Orthop.* 35 (9), 1421–1426. doi:10.1007/s00264-010-1100-y

Hunter, C. A., and Jones, S. A. (2015). IL-6 as a keystone cytokine in health and disease. *Nat. Immunol.* 16 (5), 448–457. doi:10.1038/ni.3153

Hunter, D. J., and Bierma-Zeinstra, S. (2019). Osteoarthritis. *Lancet* 393 (10182), 1745–1759. doi:10.1016/s0140-6736(19)30417-9

Hunter, D. J., Schofield, D., and Callander, E. (2014). The individual and socioeconomic impact of osteoarthritis. *Nat. Rev. Rheumatol.* 10 (7), 437–441. doi:10.1038/nrrheum.2014.44

Husa, M., Liu-Bryan, R., and Terkeltaub, R. (2010). Shifting HIFs in osteoarthritis. *Nat. Med.* 16 (6), 641–644. doi:10.1038/nm0610-641

Jia, S., Yang, Y., Bai, Y., Wei, Y., Zhang, H., Tian, Y., et al. (2022). Mechanical stimulation protects against chondrocyte pyroptosis through irisin-induced suppression of PI3K/Akt/NF-κB signal pathway in osteoarthritis. *Front. Cell Dev. Biol.* 10, 797855. doi:10.3389/fcell.2022.797855

Jones, S. A., and Jenkins, B. J. (2018). Recent insights into targeting the IL-6 cytokine family in inflammatory diseases and cancer. *Nat. Rev. Immunol.* 18 (12), 773–789. doi:10.1038/s41577-018-0066-7

Jung, T. W., Lee, S. H., Kim, H. C., Bang, J. S., Abd El-Aty, A. M., Hacımüftüoğlu, A., et al. (2018). METRNL attenuates lipid-induced inflammation and insulin resistance via AMPK or PPARδ-dependent pathways in skeletal muscle of mice. *Exp. Mol. Med.* 50 (9), 122–211. doi:10.1038/s12276-018-0147-5

Katz, J. N., Arant, K. R., and Loeser, R. F. (2021). Diagnosis and treatment of hip and knee osteoarthritis: a review. *Jama* 325 (6), 568–578. doi:10.1001/jama.2020.22171

Kelly, K. M., Smith, J. A., and Mezuk, B. (2021). Depression and interleukin-6 signaling: a Mendelian Randomization study. *Brain Behav. Immun.* 95, 106–114. doi:10.1016/j.bbi.2021.02.019

Kim, B. H., Bae, H. C., Wang, S. Y., Jang, B. S., Chang, J. H., Chie, E. K., et al. (2022). Low-dose irradiation could mitigate osteoarthritis progression via anti-inflammatory action that modulates mitochondrial function. *Radiother. Oncol.* 170, 231–241. doi:10.1016/j.radonc.2022.02.039

Kim, H., Wrann, C. D., Jedrychowski, M., Vidoni, S., Kitase, Y., Nagano, K., et al. (2018). Irisin mediates effects on bone and fat via alphaV integrin receptors. *Cell* 175 (7), 1756–1768 e1717. doi:10.1016/j.cell.2018.10.025

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 (9), 519–542. doi:10.1038/s41585-021-00476-y

Kim, T. H., Chang, J. S., Park, K. S., Park, J., Kim, N., Lee, J. I., et al. (2017). Effects of exercise training on circulating levels of Dickkpof-1 and secreted frizzled-related protein-1 in breast cancer survivors: a pilot single-blind randomized controlled trial. *PLoS One* 12 (2), e0171771. doi:10.1371/journal.pone.0171771

Klein, A. B., Nicolaisen, T. S., Ørtenblad, N., Gejl, K. D., Jensen, R., Fritzen, A. M., et al. (2021). Pharmacological but not physiological GDF15 suppresses feeding and the motivation to exercise. *Nat. Commun.* 12 (1), 1041. doi:10.1038/s41467-021-21309-x

Klosowska, K., Volin, M. V., Huynh, N., Chong, K. K., Halloran, M. M., and Woods, J. M. (2009). Fractalkine functions as a chemoattractant for osteoarthritis synovial fibroblasts and stimulates phosphorylation of mitogen-activated protein kinases and Akt. *Clin. Exp. Immunol.* 156 (2), 312–319. doi:10.1111/j.1365-2249.2009.03903.x

Kurdiova, T., Balaz, M., Vician, M., Maderova, D., Vlcek, M., Valkovic, L., et al. (2014). Effects of obesity, diabetes and exercise on Fndc5 gene expression and irisin release in human skeletal muscle and adipose tissue: *in vivo* and *in vitro* studies. *J. Physiol.* 592 (5), 1091–1107. doi:10.1113/jphysiol.2013.264655

Kurz, E., Hirsch, C. A., Dalton, T., Shadaloey, S. A., Khodadadi-Jamayran, A., Miller, G., et al. (2022). Exercise-induced engagement of the IL-15/IL-15Rα axis promotes anti-tumor immunity in pancreatic cancer. *Cancer Cell* 40 (7), 720–737.e5. doi:10.1016/j.ccell.2022.05.006

Latourte, A., Kloppenburg, M., and Richette, P. (2020). Emerging pharmaceutical therapies for osteoarthritis. *Nat. Rev. Rheumatol.* 16 (12), 673–688. doi:10.1038/s41584-020-00518-6

Law, Y. Y., Lin, Y. M., Liu, S. C., Wu, M. H., Chung, W. H., Tsai, C. H., et al. (2020). Visfatin increases ICAM-1 expression and monocyte adhesion in human osteoarthritis synovial fibroblasts by reducing miR-320a expression. *Aging (Albany NY)* 12 (18), 18635–18648. doi:10.18632/aging.103889

Lee, S., Norheim, F., Langleite, T. M., Gulseth, H. L., Birkeland, K. I., and Drevon, C. A. (2019). Effects of long-term exercise on plasma adipokine levels and inflammation-related gene expression in subcutaneous adipose tissue in sedentary dysglycaemic, overweight men and sedentary normoglycaemic men of healthy weight. *Diabetologia* 62 (6), 1048–1064. doi:10.1007/s00125-019-4866-5

Lee, U., Stuelsatz, P., Karaz, S., McKellar, D. W., Russeil, J., Deak, M., et al. (2022). A Tead1-Apelin axis directs paracrine communication from myogenic to endothelial cells in skeletal muscle. *iScience* 25 (7), 104589. doi:10.1016/j.isci.2022.104589

Leonov, A., Trofimov, S., Ermakov, S., and Livshits, G. (2011). Quantitative genetic study of amphiregulin and fractalkine circulating levels--potential markers of arthropathies. *Osteoarthr. Cartil.* 19 (6), 737–742. doi:10.1016/j.joca.2011.02.013

Li, C., Chen, K., Kang, H., Yan, Y., Liu, K., Guo, C., et al. (2017). Double-stranded RNA released from damaged articular chondrocytes promotes cartilage degeneration via Toll-like receptor 3-interleukin-33 pathway. *Cell Death Dis.* 8 (11), e3165. doi:10.1038/cddis.2017.534

Li, W., Alahdal, M., Deng, Z., Liu, J., Zhao, Z., Cheng, X., et al. (2020). Molecular functions of FSTL1 in the osteoarthritis. *Int. Immunopharmacol.* 83, 106465. doi:10.1016/j.intimp.2020.106465

Li, X., Zhu, X., Wu, H., Van Dyke, T. E., Xu, X., Morgan, E. F., et al. (2021). Roles and mechanisms of irisin in attenuating pathological features of osteoarthritis. *Front. Cell Dev. Biol.* 9, 703670. doi:10.3389/fcell.2021.703670

Liew, F. Y., Girard, J. P., and Turnquist, H. R. (2016). Interleukin-33 in health and disease. *Nat. Rev. Immunol.* 16 (11), 676–689. doi:10.1038/nri.2016.95

Liu, J., Jia, S., Yang, Y., Piao, L., Wang, Z., Jin, Z., et al. (2022). Exercise induced meteorin-like protects chondrocytes against inflammation and pyroptosis in osteoarthritis by inhibiting PI3K/Akt/NF-κB and NLRP3/caspase-1/GSDMD signaling. *Biomed. Pharmacother.* 158, 114118. doi:10.1016/j.biopha.2022.114118

Liu, M., and Hu, C. (2012). Association of MIF in serum and synovial fluid with severity of knee osteoarthritis. *Clin. Biochem.* 45 (10-11), 737–739. doi:10.1016/j.clinbiochem.2012.03.012

Long, D., Blake, S., Song, X. Y., Lark, M., and Loeser, R. F. (2008). Human articular chondrocytes produce IL-7 and respond to IL-7 with increased production of matrix metalloproteinase-13. *Arthritis Res. Ther.* 10 (1), R23. doi:10.1186/ar2376

Long, H., Liu, Q., Yin, H., Wang, K., Diao, N., Zhang, Y., et al. (2022). Prevalence trends of site-specific osteoarthritis from 1990 to 2019: findings from the global burden of disease study 2019. *Arthritis Rheumatol.* 74 (7), 1172–1183. doi:10.1002/art.42089

Lou, J., Wu, J., Feng, M., Dang, X., Wu, G., Yang, H., et al. (2022). Exercise promotes angiogenesis by enhancing endothelial cell fatty acid utilization via liver-derived extracellular vesicle miR-122-5p. *J. Sport Health Sci.* 11 (4), 495–508. doi:10.1016/j.jshs.2021.09.009

Loughlin, J. (2005). The genetic epidemiology of human primary osteoarthritis: current status. *Expert Rev. Mol. Med.* 7 (9), 1–12. doi:10.1017/s1462399405009257

Lu, Q. B., Ding, Y., Liu, Y., Wang, Z. C., Wu, Y. J., Niu, K. M., et al. (2022). Metrnl ameliorates diabetic cardiomyopathy via inactivation of cGAS/STING signaling dependent on LKB1/AMPK/ULK1-mediated autophagy. *J. Adv. Res.* 51, 161–179. doi:10.1016/j.jare.2022.10.014

Luo, J., Liu, W., Feng, F., and Chen, L. (2021). Apelin/APJ system: a novel therapeutic target for locomotor system diseases. *Eur. J. Pharmacol.* 906, 174286. doi:10.1016/j.ejphar.2021.174286

Maak, S., Norheim, F., Drevon, C. A., and Erickson, H. P. (2021). Progress and challenges in the biology of FNDC5 and irisin. *Endocr. Rev.* 42 (4), 436–456. doi:10.1210/endrev/bnab003

Macdonald, I. J., Liu, S. C., Huang, C. C., Kuo, S. J., Tsai, C. H., and Tang, C. H. (2019). Associations between adipokines in arthritic disease and implications for obesity. *Int. J. Mol. Sci.* 20 (6), 1505. doi:10.3390/ijms20061505

Martinez-Huenchullan, S. F., Tam, C. S., Ban, L. A., Ehrenfeld-Slater, P., McLennan, S. V., and Twigg, S. M. (2020). Skeletal muscle adiponectin induction in obesity and exercise. *Metabolism* 102, 154008. doi:10.1016/j.metabol.2019.154008

Mobasheri, A. (2012). Osteoarthritis year 2012 in review: biomarkers. *Osteoarthr. Cartil.* 20 (12), 1451–1464. doi:10.1016/j.joca.2012.07.009

Moon, H. Y., Song, P., Choi, C. S., Ryu, S. H., and Suh, P. G. (2013). Involvement of exercise-induced macrophage migration inhibitory factor in the prevention of fatty liver disease. *J. Endocrinol.* 218 (3), 339–348. doi:10.1530/joe-13-0135

Nasi, S., So, A., Combes, C., Daudon, M., and Busso, N. (2016). Interleukin-6 and chondrocyte mineralisation act in tandem to promote experimental osteoarthritis. *Ann. Rheum. Dis.* 75 (7), 1372–1379. doi:10.1136/annrheumdis-2015-207487

Neumann, E., Junker, S., Schett, G., Frommer, K., and Muller-Ladner, U. (2016). Adipokines in bone disease. *Nat. Rev. Rheumatol.* 12 (5), 296–302. doi:10.1038/nrrheum.2016.49

Nguyen, H. N., Noss, E. H., Mizoguchi, F., Huppertz, C., Wei, K. S., Watts, G. F. M., et al. (2017). Autocrine loop involving IL-6 family member LIF, LIF receptor, and STAT4 drives sustained fibroblast production of inflammatory mediators. *Immunity* 46 (2), 220–232. doi:10.1016/j.immuni.2017.01.004

Ni, S., Miao, K., Zhou, X., Xu, N., Li, C., Zhu, R., et al. (2015). The involvement of follistatin-like protein 1 in osteoarthritis by elevating NF-κB-mediated inflammatory cytokines and enhancing fibroblast like synoviocyte proliferation. *Arthritis Res. Ther.* 17 (1), 91. doi:10.1186/s13075-015-0605-6

Omosule, C. L., and Phillips, C. L. (2021). Deciphering myostatin's regulatory, metabolic, and developmental influence in skeletal diseases. *Front. Genet.* 12, 662908. doi:10.3389/fgene.2021.662908

Paris, M. T., Bell, K. E., and Mourtzakis, M. (2020). Myokines and adipokines in sarcopenia: understanding cross-talk between skeletal muscle and adipose tissue and the role of exercise. *Curr. Opin. Pharmacol.* 52, 61–66. doi:10.1016/j.coph.2020.06.003

Pasold, J., Osterberg, A., Peters, K., Taipaleenmäki, H., Säämänen, A. M., Vollmar, B., et al. (2013). Reduced expression of Sfrp1 during chondrogenesis and in articular chondrocytes correlates with osteoarthritis in STR/ort mice. *Exp. Cell Res.* 319 (5), 649–659. doi:10.1016/j.yexcr.2012.12.012

Pedersen, B. K. (2011). Exercise-induced myokines and their role in chronic diseases. *Brain Behav. Immun.* 25 (5), 811–816. doi:10.1016/j.bbi.2011.02.010

Pedersen, B. K. (2019). Physical activity and muscle-brain crosstalk. *Nat. Rev. Endocrinol.* 15 (7), 383–392. doi:10.1038/s41574-019-0174-x

Pedersen, B. K., and Febbraio, M. A. (2008a). 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. (2008b). Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol. Rev.* 88 (4), 1379–1406. doi:10.1152/physrev.90100.2007

Perakakis, N., Upadhyay, J., Ghaly, W., Chen, J., Chrysafi, P., Anastasilakis, A. D., et al. (2018). Regulation of the activins-follistatins-inhibins axis by energy status: impact on reproductive function. *Metabolism* 85, 240–249. doi:10.1016/j.metabol.2018.05.003

Pignolo, R. J., Wang, H., and Kaplan, F. S. (2019). Fibrodysplasia ossificans progressiva (FOP): a segmental progeroid syndrome. *Front. Endocrinol. (Lausanne)* 10, 908. doi:10.3389/fendo.2019.00908

Pillon, N. J., Smith, J. A. B., Alm, P. S., Chibalin, A. V., Alhusen, J., Arner, E., et al. (2022). Distinctive exercise-induced inflammatory response and exerkine induction in skeletal muscle of people with type 2 diabetes. *Sci. Adv.* 8 (36), eabo3192. doi:10.1126/sciadv.abo3192

Plinta, R., Olszanecka-Glinianowicz, M., Drosdzol-Cop, A., Chudek, J., and Skrzypulec-Plinta, V. (2012). The effect of three-month pre-season preparatory period and short-term exercise on plasma leptin, adiponectin, visfatin, and ghrelin levels in young female handball and basketball players. *J. Endocrinol. Invest.* 35 (6), 595–601. doi:10.3275/8014

Posa, F., Zerlotin, R., Ariano, A., Cosola, M. D., Colaianni, G., Fazio, A. D., et al. (2023). Irisin role in chondrocyte 3D culture differentiation and its possible applications. *Pharmaceutics* 15 (2), 585. doi:10.3390/pharmaceutics15020585

Rao, R. R., Long, J. Z., White, J. P., Svensson, K. J., Lou, J., Lokurkar, I., et al. (2014). Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. *Cell* 157 (6), 1279–1291. doi:10.1016/j.cell.2014.03.065

Robbins, J. M., and Gerszten, R. E. (2023). Exercise, exerkines, and cardiometabolic health: from individual players to a team sport. *J. Clin. Invest.* 133 (11), e168121. doi:10.1172/JCI168121

Rodríguez-Hernández, M., Carneros, D., Núñez-Núñez, M., Coca, R., Baena, R., López-Ruiz, G. M., et al. (2022). Identification of IL-6 signalling components as predictors of severity and outcome in COVID-19. *Front. Immunol.* 13, 891456. doi:10.3389/fimmu.2022.891456

Rose-John, S. (2012). IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. *Int. J. Biol. Sci.* 8 (9), 1237–1247. doi:10.7150/ijbs.4989

Rowe, M. A., Harper, L. R., McNulty, M. A., Lau, A. G., Carlson, C. S., Leng, L., et al. (2017). Reduced osteoarthritis severity in aged mice with deletion of macrophage migration inhibitory factor. *Arthritis Rheumatol.* 69 (2), 352–361. doi:10.1002/art.39844

Scanzello, C. R., Umoh, E., Pessler, F., Diaz-Torne, C., Miles, T., Dicarlo, E., et al. (2009). Local cytokine profiles in knee osteoarthritis: elevated synovial fluid interleukin-15 differentiates early from end-stage disease. *Osteoarthr. Cartil.* 17 (8), 1040–1048. doi:10.1016/j.joca.2009.02.011

Seo, D. I., So, W. Y., Ha, S., Yoo, E. J., Kim, D., Singh, H., et al. (2011). Effects of 12 weeks of combined exercise training on visfatin and metabolic syndrome factors in obese middle-aged women. *J. Sports Sci. Med.* 10 (1), 222–226. doi:10.5932/JKPHN.2012.26.3.417

Severinsen, M. C. K., and Pedersen, B. K. (2020). Muscle-organ crosstalk: the emerging roles of myokines. *Endocr. Rev.* 41 (4), 594–609. doi:10.1210/endrev/bnaa016

Shimano, M., Ouchi, N., and Walsh, K. (2012). Cardiokines: recent progress in elucidating the cardiac secretome. *Circulation* 126 (21), e327–e332. doi:10.1161/circulationaha.112.150656

Silacci, P., Dayer, J. M., Desgeorges, A., Peter, R., Manueddu, C., and Guerne, P. A. (1998). Interleukin (IL)-6 and its soluble receptor induce TIMP-1 expression in synoviocytes and chondrocytes, and block IL-1-induced collagenolytic activity. *J. Biol. Chem.* 273 (22), 13625–13629. doi:10.1074/jbc.273.22.13625

Simpson, K. A., and Singh, M. A. (2008). Effects of exercise on adiponectin: a systematic review. *Obes. (Silver Spring)* 16 (2), 241–256. doi:10.1038/oby.2007.53

Snelling, S., Ferreira, A., and Loughlin, J. (2007). Allelic expression analysis suggests that cis-acting polymorphism of FRZB expression does not contribute to osteoarthritis susceptibility. *Osteoarthr. Cartil.* 15 (1), 90–92. doi:10.1016/j.joca.2006.06.016

Sobieh, B. H., Kassem, D. H., Zakaria, Z. M., and El-Mesallamy, H. O. (2021). Potential emerging roles of the novel adipokines adipolin/CTRP12 and meteorin-like/METRNL in obesity-osteoarthritis interplay. *Cytokine* 138, 155368. doi:10.1016/j.cyto.2020.155368

Steel, J. C., Waldmann, T. A., and Morris, J. C. (2012). Interleukin-15 biology and its therapeutic implications in cancer. *Trends Pharmacol. Sci.* 33 (1), 35–41. doi:10.1016/j.tips.2011.09.004

Stone, A. V., Vanderman, K. S., Willey, J. S., Long, D. L., Register, T. C., Shively, C. A., et al. (2015). Osteoarthritic changes in vervet monkey knees correlate with meniscus degradation and increased matrix metalloproteinase and cytokine secretion. *Osteoarthr. Cartil.* 23 (10), 1780–1789. doi:10.1016/j.joca.2015.05.020

Sun, J. M., Sun, L. Z., Liu, J., Su, B. H., and Shi, L. (2013). Serum interleukin-15 levels are associated with severity of pain in patients with knee osteoarthritis. *Dis. Markers* 35 (3), 203–206. doi:10.1155/2013/176278

Suzuki, M., Hashizume, M., Yoshida, H., Shiina, M., and Mihara, M. (2010). IL-6 and IL-1 synergistically enhanced the production of MMPs from synovial cells by up-regulating IL-6 production and IL-1 receptor I expression. *Cytokine* 51 (2), 178–183. doi:10.1016/j.cyto.2010.03.017

Talabot-Ayer, D., McKee, T., Gindre, P., Bas, S., Baeten, D. L., Gabay, C., et al. (2012). Distinct serum and synovial fluid interleukin (IL)-33 levels in rheumatoid arthritis, psoriatic arthritis and osteoarthritis. *Jt. Bone Spine* 79 (1), 32–37. doi:10.1016/j.jbspin.2011.02.011

Tang, Q., Hu, Z. C., Shen, L. Y., Shang, P., Xu, H. Z., and Liu, H. X. (2018). Association of osteoarthritis and circulating adiponectin levels: a systematic review and meta-analysis. *Lipids Health Dis.* 17 (1), 189. doi:10.1186/s12944-018-0838-x

Thornton, S., Sowders, D., Aronow, B., Witte, D. P., Brunner, H. I., Giannini, E. H., et al. (2002). DNA microarray analysis reveals novel gene expression profiles in collagen-induced arthritis. *Clin. Immunol.* 105 (2), 155–168. doi:10.1006/clim.2002.5227

Thysen, S., Luyten, F. P., and Lories, R. J. (2015). Loss of Frzb and Sfrp1 differentially affects joint homeostasis in instability-induced osteoarthritis. *Osteoarthr. Cartil.* 23 (2), 275–279. doi:10.1016/j.joca.2014.10.010

Torstensen, T. A., Østerås, H., LoMartire, R., Rugelbak, G. M., Grooten, W. J. A., and Äng, B. O. (2023). High- versus low-dose exercise therapy for knee osteoarthritis: a randomized controlled multicenter trial. *Ann. Intern Med.* 176 (2), 154–165. doi:10.7326/m22-2348

Tsuchida, A. I., Beekhuizen, M., Rutgers, M., van Osch, G. J., Bekkers, J. E., Bot, A. G., et al. (2012). Interleukin-6 is elevated in synovial fluid of patients with focal cartilage defects and stimulates cartilage matrix production in an *in vitro* regeneration model. *Arthritis Res. Ther.* 14 (6), R262. doi:10.1186/ar4107

Udomsinprasert, W., Manoy, P., Yuktanandana, P., Tanavalee, A., Anomasiri, W., and Honsawek, S. (2020). Decreased serum adiponectin reflects low vitamin D, high interleukin 6, and poor physical performance in knee osteoarthritis. *Arch. Immunol. Ther. Exp. Warsz.* 68 (3), 16. doi:10.1007/s00005-020-00580-8

Vadala, G., Di Giacomo, G., Ambrosio, L., Cannata, F., Cicione, C., Papalia, R., et al. (2020). Irisin recovers osteoarthritic chondrocytes *in vitro*. *Cells* 9 (6), 1478. doi:10.3390/cells9061478

van Loon, K., Huijbers, E. J. M., and Griffioen, A. W. (2021). Secreted frizzled-related protein 2: a key player in noncanonical Wnt signaling and tumor angiogenesis. *Cancer Metastasis Rev.* 40 (1), 191–203. doi:10.1007/s10555-020-09941-3

Vinel, C., Lukjanenko, L., Batut, A., Deleruyelle, S., Pradère, J. P., Le Gonidec, S., et al. (2018). The exerkine apelin reverses age-associated sarcopenia. *Nat. Med.* 24 (9), 1360–1371. doi:10.1038/s41591-018-0131-6

Wang, D., Day, E. A., Townsend, L. K., Djordjevic, D., Jørgensen, S. B., and Steinberg, G. R. (2021). GDF15: emerging biology and therapeutic applications for obesity and cardiometabolic disease. *Nat. Rev. Endocrinol.* 17 (10), 592–607. doi:10.1038/s41574-021-00529-7

Wang, F.-S., Kuo, C.-W., Ko, J.-Y., Chen, Y.-S., Wang, S.-Y., Ke, H.-J., et al. (2020). Irisin mitigates oxidative stress, chondrocyte dysfunction and osteoarthritis development through regulating mitochondrial integrity and autophagy. *Antioxidants* 9 (9), 810. doi:10.3390/antiox9090810

Wang, Y., Li, D., Xu, N., Tao, W., Zhu, R., Sun, R., et al. (2011). Follistatin-like protein 1: a serum biochemical marker reflecting the severity of joint damage in patients with osteoarthritis. *Arthritis Res. Ther.* 13 (6), R193. doi:10.1186/ar3522

Warner, S. C., Nair, A., Marpadga, R., Chubinskaya, S., Doherty, M., Valdes, A. M., et al. (2020). IL-15 and IL15RA in osteoarthritis: association with symptoms and protease production, but not structural severity. *Front. Immunol.* 11, 1385. doi:10.3389/fimmu.2020.01385

Watt, F. E., Hamid, B., Garriga, C., Judge, A., Hrusecka, R., Custers, R. J. H., et al. (2020). The molecular profile of synovial fluid changes upon joint distraction and is associated with clinical response in knee osteoarthritis. *Osteoarthr. Cartil.* 28 (3), 324–333. doi:10.1016/j.joca.2019.12.005

Wiegertjes, R., van de Loo, F. A. J., and Blaney Davidson, E. N. (2020). A roadmap to target interleukin-6 in osteoarthritis. *Rheumatol. Oxf.* 59 (10), 2681–2694. doi:10.1093/rheumatology/keaa248

Wojdasiewicz, P., Poniatowski Ł, A., Kotela, A., Skoda, M., Pyzlak, M., Stangret, A., et al. (2020). Comparative analysis of the occurrence and role of CX3CL1 (fractalkine) and its receptor CX3CR1 in hemophilic arthropathy and osteoarthritis. *J. Immunol. Res.* 2020, 2932696. doi:10.1155/2020/2932696

Xi, Y., Hao, M., Liang, Q., Li, Y., Gong, D. W., and Tian, Z. (2021). Dynamic resistance exercise increases skeletal muscle-derived FSTL1 inducing cardiac angiogenesis via DIP2A-Smad2/3 in rats following myocardial infarction. *J. Sport Health Sci.* 10 (5), 594–603. doi:10.1016/j.jshs.2020.11.010

Xu, C., Jiang, T., Ni, S., Chen, C., Li, C., Zhuang, C., et al. (2020). FSTL1 promotes nitric oxide-induced chondrocyte apoptosis via activating the SAPK/JNK/caspase3 signaling pathway. *Gene* 732, 144339. doi:10.1016/j.gene.2020.144339

Yang, F., Zhou, S., Wang, C., Huang, Y., Li, H., Wang, Y., et al. (2017). Epigenetic modifications of interleukin-6 in synovial fibroblasts from osteoarthritis patients. *Sci. Rep.* 7, 43592. doi:10.1038/srep43592

Yang, S., Ryu, J. H., Oh, H., Jeon, J., Kwak, J. S., Kim, J. H., et al. (2015). NAMPT (visfatin), a direct target of hypoxia-inducible factor-2α, is an essential catabolic regulator of osteoarthritis. *Ann. Rheum. Dis.* 74 (3), 595–602. doi:10.1136/annrheumdis-2013-204355

Yang, Y., Wang, Y., Kong, Y., Zhang, X., Zhang, H., Feng, X., et al. (2020). Moderate mechanical stimulation protects rats against osteoarthritis through the regulation of TRAIL via the NF-κB/NLRP3 pathway. *Oxid. Med. Cell Longev.* 2020, 6196398. doi:10.1155/2020/6196398

Yang, Y., Wang, Y., Kong, Y., Zhang, X., Zhang, H., Gang, Y., et al. (2019). Mechanical stress protects against osteoarthritis via regulation of the AMPK/NF-κB signaling pathway. *J. Cell Physiol.* 234 (6), 9156–9167. doi:10.1002/jcp.27592

Yao, Q., Wu, X., Tao, C., Gong, W., Chen, M., Qu, M., et al. (2023). Osteoarthritis: pathogenic signaling pathways and therapeutic targets. *Signal Transduct. Target Ther.* 8 (1), 56. doi:10.1038/s41392-023-01330-w

Yuan, J., Xu, B., Ma, J., Pang, X., Fu, Y., Liang, M., et al. (2023). MOTS-c and aerobic exercise induce cardiac physiological adaptation via NRG1/ErbB4/CEBPβ modification in rats. *Life Sci.* 315, 121330. doi:10.1016/j.lfs.2022.121330

Yue, L., and Berman, J. (2022). What is osteoarthritis? *Jama* 327 (13), 1300. doi:10.1001/jama.2022.1980

Zhang, H. X., Wang, Y. G., Lu, S. Y., Lu, X. X., and Liu, J. (2016a). Identification of IL-7 as a candidate disease mediator in osteoarthritis in Chinese Han population: a case-control study. *Rheumatol. Oxf.* 55 (9), 1681–1685. doi:10.1093/rheumatology/kew220

Zhang, P. L., Liu, J., Xu, L., Sun, Y., and Sun, X. C. (2016b). Synovial fluid macrophage migration inhibitory factor levels correlate with severity of self-reported pain in knee osteoarthritis patients. *Med. Sci. Monit.* 22, 2182–2186. doi:10.12659/msm.895704

Zhang, X., Wu, Y., Pan, Z., Sun, H., Wang, J., Yu, D., et al. (2016c). The effects of lactate and acid on articular chondrocytes function: implications for polymeric cartilage scaffold design. *Acta Biomater.* 42, 329–340. doi:10.1016/j.actbio.2016.06.029

Zhang, Y., Li, D., Zhu, Z., Chen, S., Lu, M., Cao, P., et al. (2022). Evaluating the impact of metformin targets on the risk of osteoarthritis: a mendelian randomization study. *Osteoarthr. Cartil.* 30 (11), 1506–1514. doi:10.1016/j.joca.2022.06.010

frontiers | Frontiers in Physiology

TYPE Brief Research Report
PUBLISHED 08 December 2023
DOI 10.3389/fphys.2023.1310319

OPEN ACCESS

EDITED BY
Simone Luti,
University of Florence, Italy

REVIEWED BY
Samuel Honório,
Polytechnic Institute of Castelo Branco, Portugal
Karin Vonbank,
Medical University of Vienna, Austria

*CORRESPONDENCE
Andrea Meloni,
andrea.meloni@unimi.it

RECEIVED 09 October 2023
ACCEPTED 27 November 2023
PUBLISHED 08 December 2023

CITATION
Meloni A, Codella R, Gotti D, Di Gennaro S, Luzi L and Filipas L (2023), Fat oxidation rates and cardiorespiratory responses during exercise in different subject populations with post-acute sequelae of SARS-CoV-2 infection: a comparison with normative percentile values.
Front. Physiol. 14:1310319.
doi: 10.3389/fphys.2023.1310319

Fat oxidation rates and cardiorespiratory responses during exercise in different subject populations with post-acute sequelae of SARS-CoV-2 infection: a comparison with normative percentile values

Andrea Meloni[1,2]*, Roberto Codella[1,2], Daniel Gotti[1], Simone Di Gennaro[3], Livio Luzi[1,2] and Luca Filipas[1,2]

[1]Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy, [2]Department of Endocrinology, Nutrition and Metabolic Diseases, IRCCS MultiMedica, Milan, Italy, [3]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics and Mother-Child Sciences, Università degli Studi di Genova, Genova, Italy

Introduction: Post-acute sequelae of SARS-CoV-2 infection (PASC) presents a spectrum of symptoms following acute COVID-19, with exercise intolerance being a prevalent manifestation likely linked to disrupted oxygen metabolism and mitochondrial function. This study aims to assess maximal fat oxidation (MFO) and exercise intensity at MFO (FATmax) in distinct PASC subject groups and compare these findings with normative data.

Methods: Eight male subjects with PASC were involved in this study. The participants were divided into two groups: "endurance-trained" subjects ($\dot{V}O_2$ max > 55 mL/min/kg) and "recreationally active" subjects ($\dot{V}O_2$ max < 55 mL/min/kg). Each subject performed a graded exercise test until maximal oxygen consumption ($\dot{V}O_2$ max) to measure fat oxidation. Subsequently, MFO was assessed, and FATmax was calculated as the ratio between $\dot{V}O_2$ at MFO and $\dot{V}O_2$ max.

Results: The MFO and FATmax of "endurance-trained" subjects were 0.85, 0.89, 0.71, and 0.42 and 68%, 69%, 64%, and 53%, respectively. Three out of four subjects showed both MFO and FATmax values placed over the 80th percentile of normative data. The MFO and FATmax of "recreationally active" subjects were 0.34, 0.27, 0.35, and 0.38 and 47%, 39%, 43%, and 41%, respectively. All MFO and FATmax values of those subjects placed below the 20th percentile or between the 20th and 40th percentile.

Discussion: Significant differences in MFO and FATmax values between 'endurance-trained' and "recreationally active" subjects suggest that specific

endurance training, rather than simply an active lifestyle, may provide protective effects against alterations in mitochondrial function during exercise in subjects with PASC.

KEYWORDS

post-acute sequelae of SARS-CoV-2 infection, fat oxidation, metabolic dysfunction, cycling, exercise performance

Introduction

The syndrome of post-acute sequelae of SARS-CoV-2 infection (PASC) is a clinical condition characterized by a wide range of symptoms for 4 weeks or more following acute COVID-19 (World Health Organization, 2023). Exercise intolerance is one of the most widespread manifestations among subjects with PASC. This condition may be the result of impaired oxygen metabolic homeostasis and altered mitochondrial function (Astin et al., 2023). In general, mitochondrial disorders are a complex group of diseases caused by impairment of the mitochondrial respiratory chain (or electron transport chain), which in some patients can lead to an unexplained post-viral illness and myalgic encephalomyelitis/chronic fatigue syndrome (Wood et al., 2021). From a biochemical perspective, mitochondria may utilize various substrates depending on the load and duration of exercise (McArdle WD et al., 2001). During exercise lasting more than 1 minute, adenosine triphosphate (ATP) production is mainly generated via oxidative phosphorylation in the tricarboxylic acid cycle (TCA). The substrates utilized in the TCA to generate reducing equivalents fuel the electron transport chain inside mitochondria are fatty acids (FAs) and carbohydrates (CAs). In this energy production system, called the aerobic system, oxygen (O_2) represents the final electron acceptor in mitochondria (McArdle WD et al., 2001). During a submaximal exercise, FA and CA are the main substrates to produce energy, and their contribution depends on exercise intensity. In this sense, rates of b-oxidation of FA (FATox rate) and CA oxidation rates (CHOox rate) are inversely proportional throughout an incremental exercise. Specifically, after reaching the maximal rate of fat oxidation (MFO), throughout an incremental test, the FATox rate tends to decrease, while the CHOox rate tends to increase. Thus, a more detailed analysis in the plasma of metabolites involved in the aerobic system was conducted recently in order to detect the presence of a metabolic dysfunction (Guntur et al., 2022). Results showed that the plasma of PASC subjects exhibited significantly higher levels of acyl-carnitines and free FAs and lower levels of pyruvate, lactate, and TCA metabolites such as succinate, malate, and citrate, compared to an healthy group and a group of subjects recovered from COVID-19, without PASC. These data are indicative of ongoing mobilization of FA but show impaired ability for oxidation due to mitochondrial dysfunction. In this contest, other research groups (de Boer et al., 2022) investigated whether patients with PASC had compromised mitochondrial function during graded exercise. A cardiopulmonary exercise test (CPET) has been used to calculate the FATox rate and lactate clearance, providing insight into mitochondrial function. Results showed inappropriately high arterial lactate levels and reduced FATox rate at relatively low exercise intensity in this type of subjects. Those data indicate that the transition from the FATox rate to the CHOox rate occurs earlier, suggesting, also in this case, dysfunctional mitochondria. Specifically, the premature lactate accumulation suggests either a metabolic shift in increased glycolysis or the inability to utilize lactate in the mitochondria as an alternative source of energy during exercise (de Boer et al., 2022). Normally, during an incremental exercise, lactate values and oxygen consumption ($\dot{V}O_2$) increase, and the substrate contribution (FA and CA) can be estimated through the assessment of $\dot{V}O_2$ and carbon dioxide production ($\dot{V}O_2$) (McArdle WD et al., 2001). Specifically, the FATox rate can be calculated by the stoichiometric equation (Frayn, 1983). Indeed, a well-trained endurance athlete is well known to have a high MFO and a declining FATox rate at exercise intensities of approximately 80%–85% of maximal oxygen consumption ($\dot{V}O_2$ max) (Hurley et al., 1986; Martin et al., 1993; Phillips et al., 1996; Bircher and Knetchle, 2004). Moreover, fat oxidation capacity has been correlated with performance in ironman triathletes, which take part in ultra-endurance events (Frandsen et al., 2017). A recent review showed normative percentile values for MFO and FATmax in different subject populations in order to contextualize individually measured values and define the fat oxidation capacity of given research cohorts (Maunder et al., 2018). Given these findings, the objective of this study is to assess MFO and FATmax in two distinct populations of individuals with PASC and to compare these data with the normative percentile values from corresponding healthy cohorts. This analysis aims to determine whether the chronic effects of virus infection may impair FA metabolism during exercise in different subgroups of the PASC population.

Materials and methods

Eight male subjects were involved in this study. The investigation lasted 3 months, and it was a pilot study that will serve as a basis for future more in-depth research on the topic at hand. Following the classification of a recent review (Maunder et al., 2018), regarding normative values of MFO and FATmax, subjects were divided into two groups: “endurance-trained” and “recreationally active.” “Endurance-trained” was defined as a subject with VO_2max >55 mL/min/kg and active engagement in training for endurance events. “Recreationally active” was defined as physically active, not training for specific endurance events, and with VO_2max <55 mL/min/kg (Maunder et al., 2018). Specifically, recreationally active subjects were involved in activities such as tennis, soccer, and gym fitness. Their anthropometric, physiological, and training characteristics are reported in Table 1. Before viral infection, both groups had been maintaining the same training or physical activity status compared to the post-COVID-19 period. The subjects had been reported a positive diagnosis of SARS-CoV-2 within

10.3389/fphys.2023.1310319

TABLE 1 Anthropometric, physiological, and training characteristics for each group. Data are reported as mean values ±SD.

Group	Age	Height (cm)	Weight (kg)	BMI (kg/m^2)	$\dot{V}O_2$ max (mL/min/kg)	Endurance training (hours/week)	General physical activity (hours/week)
Endurance-trained	36.3 ± 15.3	175.8 ± 5.1	65.3 ± 7.6	21.1 ± 2.0	69.8 ± 14.9	14.3 ± 5.9	—
Recreationally active	26.5 ± 8.5	179.0 ± 8.2	74.1 ± 3.5	23.7 ± 1.8	47.5 ± 4.7	—	3 ± 1.4

TABLE 2 MFO and FATmax values and relative percentile for each subject.

	Subject	$\dot{V}O_2$ max (mL/min/kg)	MFO	Relative percentile	FATmax (% $\dot{V}O_2$ max)	Relative percentile
Endurance-trained	1	81.2	0.85	>80th	68%	>80th
	2	84.1	0.89	>80th	69%	>80th
	3	56.7	0.71	>80th	64%	>80th
	4	57.0	0.42	20th–40th	53%	40th–60th
Recreationally active	5	41.0	0.34	20th	47%	20th–40th
	6	47.9	0.27	<20th	39%	<20th
	7	52.2	0.35	20th–40th	43%	<20th
	8	48.8	0.38	20th–40th	41%	<20th

12 months before, with mild symptoms and no need for hospital care. The therapy was limited to taking NSAIDs for a few days. The subjects suffered PASC with preserved pulmonary and cardiac function and presented brain fog, insomnia, and memory impairment with the main symptoms. In order to detect the MFO and FATmax, each subject performed the "FATmax test" (Achten and Jeukendrup, 2004), a graded exercise test on an indoor roller (Direto XR-T, ELITE), with their own bike. Workload roller, expressed in watt (W), was monitored by software My E-Training, ELITE. Pedaling cadence was freely chosen and maintained constant (± five repetitions per minute (rpm)). Each participant completed the test at the same time of the day (±2 h) after 7–8 h of sleep and under similar environmental conditions (18–20 °C). Participants were asked to consume the same meals and drinks during the 24 h prior to testing and to fast overnight before the test. Specifically, after a free warm-up period of 5 min, the subjects performed a continuous ramp test for $\dot{V}O_2$ max, starting at 50 W and increasing 35 W every 2 min. At the end of each step, lactate was collected through a capillary blood sample. Each participant gave written informed consent prior to the study. Study procedures were conducted in accordance with the Declaration of Helsinki for experimentation on human participants. The study was approved by the Ethics Committee of the University of Milan (approval no. 52/20, attachment 4).

Gas exchange and lactate measurements

Expiratory ventilation (VE), $\dot{V}O_2$, and $\dot{V}CO_2$ were analyzed using a portable metabolimeter (K5, COSMED). The highest 15-s VO2 was considered for the individual $\dot{V}O_2$ max assessment (Midgley et al., 2006; 2007). Lactate levels were determined by using a portable lactacidometer (Lactate Pro 2) through a capillary blood sample collected from the earlobe. Heart rate was monitored during the test with a heart rate sensor associated to the roller software (Polar H10; Polar Electro, Kempele, Finland). $\dot{V}O_2$ values were converted to associate power outputs by the linear regression of the $\dot{V}O_2$ vs. power output relationship using the last minute $\dot{V}O_2$ data in each incremental stage. For calculations of the total FATox rate during exercise, the stoichiometric equations by Frayn (1983) were used:

$$\text{FATox}\left[\frac{g}{min}\right] = 1.673 \times \dot{V}O_2\left[\frac{L}{min}\right] - 1.673 \times \dot{V}CO_2\left[\frac{L}{min}\right].$$

FATox rates and $\dot{V}O_2$ for each step were assessed using the last minute $\dot{V}O_2$ data in each incremental step. Thus, MFO and FATmax, seen as the ratio between $\dot{V}O_2$ at MFO and $\dot{V}O_2$ max, were calculated. Furthermore, lactate kinetics throughout the test, until the workload step with the lowest FATox rate value, was determined for each subject. Thus, lactate values at MFO for each subject were reported.

Results

MFO and FATmax values for "endurance-trained" and "recreationally active" subjects are summarized in Table 2. The subjects' FATox rate for each step is described in Figure 1. Lactate kinetics until the workload step with the lowest FATox rate is described in Figure 2. The MFO and FATmax of "endurance-trained" subjects were 0.85, 0.89, 0.71, and 0.42 and 68%, 69%, 64%, and 53%, respectively. Subjects 1, 2, and 3 showed high MFO and FATmax, and those values both placed over the highest

10.3389/fphys.2023.1310319

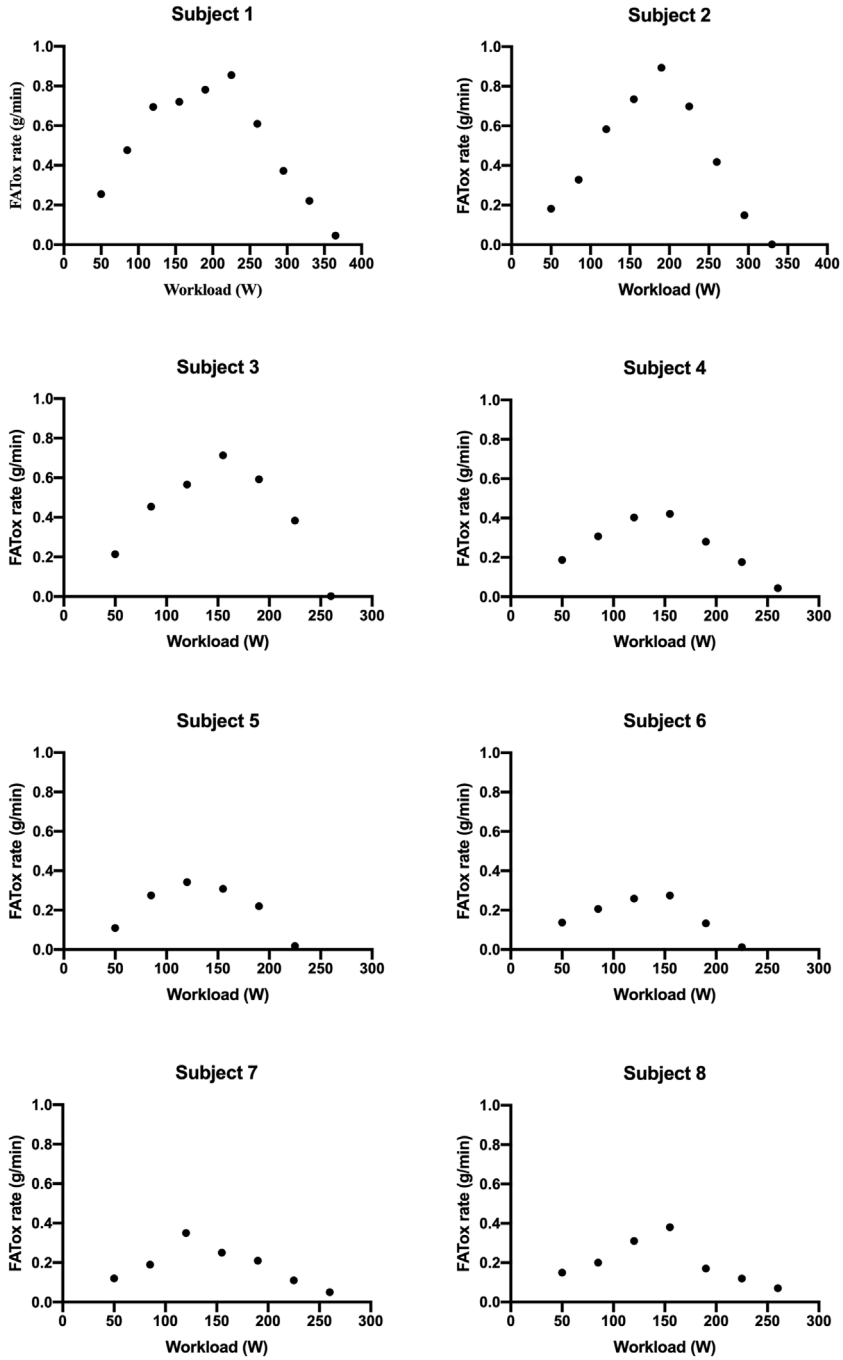


FIGURE 1
Subjects' FATox rates for each step of the "FATmax test."

percentile (80th percentile) of normative data. MFO and FATmax of subject 4 placed between the 20th and 40th percentile of MFO normative data and between the 40th and 60th percentile of FATmax normative data. The MFO and FATmax of "recreationally active" subjects were 0.34, 0.27, 0.35, and 0.38 and 47%, 39%, 43%, and 41%, respectively. MFO and FATmax of subject 5 placed on the 20th percentile and between the 20th and 40th percentile, respectively. MFO and FATmax of subject 6 both placed below the 20th percentile. MFO and FATmax of subject 7 placed between the 20th and 40th percentile and below the 20th percentile, respectively. MFO and FATmax of subject 8 placed between the 20th and 40th percentile and below the 20th percentile, respectively. In the four "endurance-trained" subjects, lactate values at MFO were 1.5, 1.8, 2.1, and 1.5, respectively. In the four "recreationally active" subjects, lactate values at MFO were 1.8, 2.5, 2.3, and 2.8, respectively.

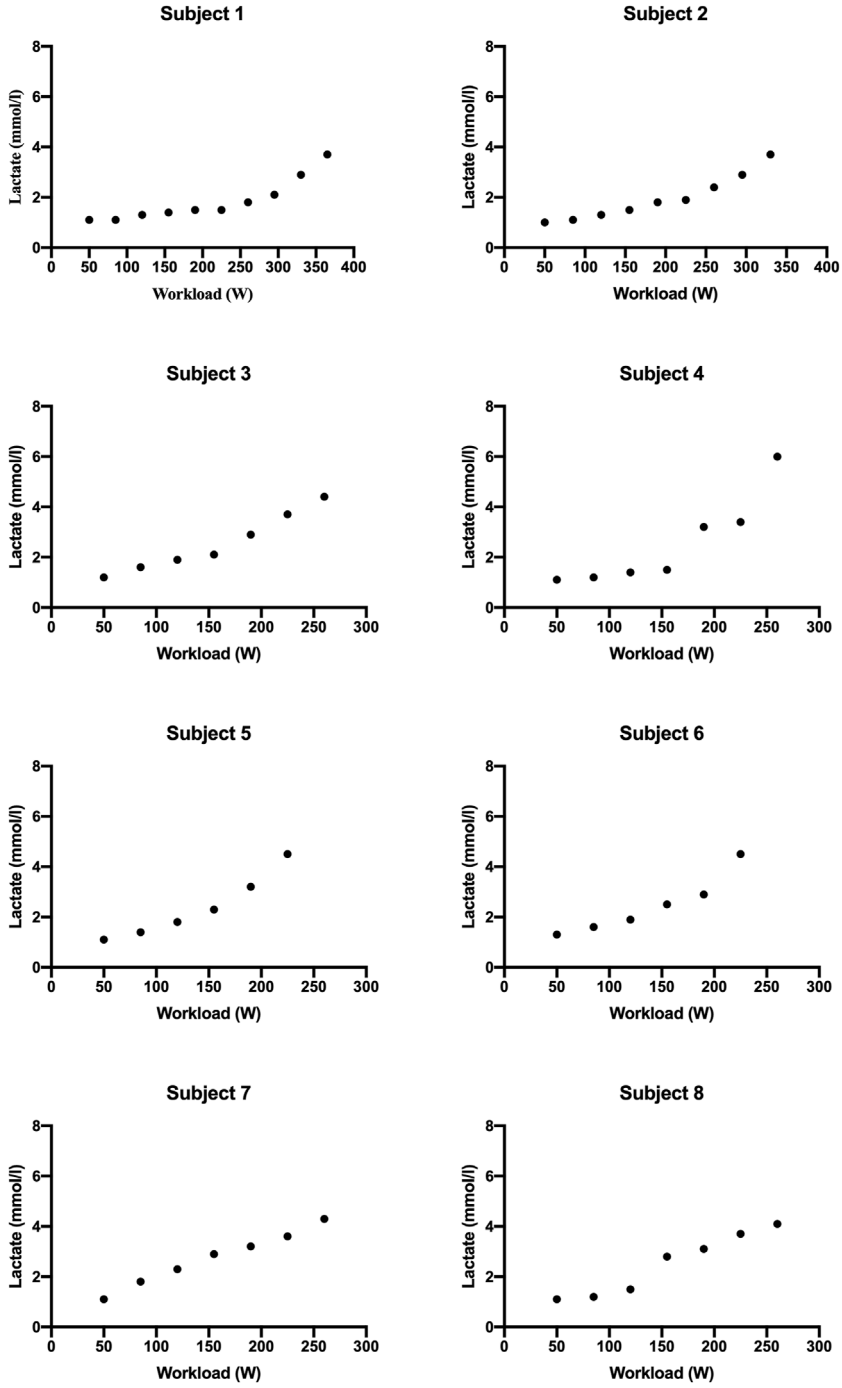


FIGURE 2
Lactate values for each subject until workload step with the lowest FATox rate.

Discussion

PASC presents a spectrum of symptoms following acute COVID-19, with exercise intolerance being a prevalent manifestation likely linked to disrupted oxygen metabolism and mitochondrial function (Astin et al., 2023). Specifically, it seems that metabolic disfunction leads to a reduction of the FATox rate at relatively low exercise (de Boer et al., 2022). At the same time, it is well known that endurance training stimulates massive FA mobilization, and in this way, it is capable to improve both MFO and FATmax. Thus, the aim of this study is to analyze MFO and FATmax in different types of subjects with PASC and compare them with normative percentile values. All subjects presented mild symptoms of PASC with preserved pulmonary and cardiac function; thus, they were able to conduct a regular daily work activity. A recent review described normative data for different subject populations, including "endurance-trained," engaged in training for endurance events, and "recreationally active," physically active but not trained for endurance events

(Maunder et al., 2018). In this context, this study showed how MFO and FATmax of subjects 3 from 4 of "endurance-trained" participants placed over the 80th percentile of their corresponding cohort. Only one "endurance-trained" subject presented MFO and FATmax values below the 60th percentile. These data could be the consequence of the infection of the subject by the virus two times within 6 months. Meanwhile, all "recreationally active" subjects showed MFO and FATmax values placed below the 40th percentile of their corresponding population. From lactate kinetics analysis, it appears how "endurance-trained" subjects show, generally, constant low values throughout the first steps of the test. Meanwhile, in the "recreationally active" group, there emerges, since the early steps, a more linear correlation between lactate values and workload. Indeed, lactate values at MFO in "endurance-trained" subjects were tendentially lower than in "recreationally active" subjects. These data strengthen the hypothesis that specific endurance training improves FA oxidation at relatively low exercise intensity, delaying, in this way, the increase of CHOox and, consequently, the accumulation of blood lactate. Taking into account all these preliminary findings, it could be suggestive of assuming that a training focus on endurance capacity may offer greater protection, compared to other types of exercise, against alterations caused by viral infection, such as mitochondrial dysfunction or, more broadly, anomalies within the pathway of FA oxidation.

Limitations of this study and perspectives

Due to the absence of the subjects' physiological data prior to SARS-CoV-2 infection, it was not possible comparing metabolic variables before and after infection in this preliminary report. In the future, several studies will be necessary to contribute to a deeper understanding of the relationship between exercise, mitochondrial function, and metabolic dysregulation in PASC subjects. Specifically, it might be useful to design a longitudinal study to investigate the effects of a structured exercise intervention, focusing on endurance training, on individuals with PASC and assess changes in exercise tolerance, mitochondrial function, and metabolic parameters over time. This study could include subgroups with varying exercise intensities and durations to determine the optimal training regimen for improving post-viral exercise intolerance in PASC patients. Additionally, it would be necessary conducting a comprehensive study to analyze metabolic profiles and mitochondrial function in different subtypes of PASC. Through this investigation, it will be possible to understand whether there are distinct metabolic signatures associated with varying symptomatology within PASC. This could involve advanced metabolomics profiling, including plasma metabolites and mitochondrial biomarkers, to identify specific metabolic dysfunctions in subgroups of PASC patients and tailor interventions accordingly. Finally, it could be interesting to compare the impact of different exercise modalities, such as aerobic exercise, resistance training, and a combination of both, on the metabolic and mitochondrial function as well. In this way, considerations for individualized exercise prescriptions based on the severity and nature of PASC symptoms would be valuable in developing targeted rehabilitation strategies.

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 humans were approved by the Ethics Committee of the University of Milan. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

AM: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, writing–original draft, and writing–review and editing. RC: data curation, formal analysis, investigation, supervision, and writing–review and editing. DG: data curation, formal analysis, investigation, software, visualization, and writing–review and editing. SD: data curation, formal analysis, investigation, software, visualization, and writing–review and editing. LL: formal analysis, investigation, methodology, supervision, visualization, and writing–review and editing. LF: conceptualization, formal analysis, investigation, methodology, visualization, writing–original draft, and writing–review and editing.

Funding

The authors declare financial support was received for the publication of this article. This work has been supported by the Italian Ministry of Health–Ricerca Corrente–IRCCS MultiMedica.

Acknowledgments

The authors thank the 'Fondazione Romeo ed Enrica Invernizzi' for supporting AM.

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.

Publisher's note

References

Achten, J., and Jeukendrup, A. E. (2004). Relation between plasma lactate concentration and fat oxidation rates over a wide range of exercise intensities. *Int. J. Sports Med.* 25, 32–37. doi:10.1055/s-2003-45231

Astin, R., Banerjee, A., Baker, M. R., Dani, M., Ford, E., Hull, J. H., et al. (2023). Long COVID: mechanisms, risk factors and recovery. *Exp. Physiol.* 108, 12–27. doi:10.1113/EP090802

Bircher, S., and Knetchle, B. (2004). Relation between plasma lactate concentration and fat oxidation rates over a wide range of exercise intensities. *Int. J. Sports Med.* 25, 32–37. doi:10.1055/s-2003-45231

de Boer, E., Petrache, I., Goldstein, N. M., Olin, J. T., Keith, R. C., Modena, B., et al. (2022). Decreased fatty acid oxidation and altered lactate production during exercise in patients with post-acute COVID-19 syndrome. *Am. J. Respir. Crit. Care Med.* 205, 126–129. doi:10.1164/rccm.202108-1903LE

Frandsen, J., Vest, S., Larsen, S., Dela, F., and Helge, J. (2017). Maximal fat oxidation is related to performance in an ironman triathlon. *Int. J. Sports Med.* 38, 975–982. doi:10.1055/s-0043-117178

Frayn, K. N. (1983). Calculation of substrate oxidation rates *in vivo* from gaseous exchange. *J. Appl. Physiol.* 55, 628–634. doi:10.1152/jappl.1983.55.2.628

Guntur, V. P., Nemkov, T., de Boer, E., Mohning, M. P., Baraghoshi, D., Cendali, F. I., et al. (2022). Signatures of mitochondrial dysfunction and impaired fatty acid metabolism in plasma of patients with post-acute sequelae of COVID-19 (PASC). *Metabolites* 12, 1026. doi:10.3390/metabo12111026

Hurley, B. F., Nemeth, P. M., Martin, W. H., Hagberg, J. M., Dalsky, G. P., and Holloszy, J. O. (1986). Muscle triglyceride utilization during exercise: effect of training. *J. Appl. Physiol.* 60, 562–567. doi:10.1152/jappl.1986.60.2.562

Martin, W. H., Dalsky, G. P., Hurley, B. F., Matthews, D. E., Bier, D. M., Hagberg, J. M., et al. (1993). Effect of endurance training on plasma free fatty acid turnover and oxidation during exercise. *Am. J. Physiology-Endocrinology Metabolism* 265, E708–E714. doi:10.1152/ajpendo.1993.265.5.E708

Maunder, E., Plews, D. J., and Kilding, A. E. (2018). Contextualising maximal fat oxidation during exercise: determinants and normative values. *Front. Physiol.* 9, 599. doi:10.3389/fphys.2018.00599

McArdle, W. D., Katch, F. I., and Katch, V. L. (2001). *Exercise Physiology: energy, nutrition, and human performance*. 5th ed. Philadelphia: Lippincott Williams and Wilkins.

Midgley, A. W., McNaughton, L. R., Polman, R., and Marchant, D. (2007). Criteria for determination of maximal oxygen uptake: a brief critique and recommendations for future research. *Sports Med.* 37, 1019–1028. doi:10.2165/00007256-200737120-00002

Midgley, A. W., Mc Naughton, L. R., and Wilkinson, M. (2006). Criteria and other methodological considerations in the evaluation of time at V.O2max. *J. Sports Med. Phys. Fit.* 46, 183–188.

Phillips, S. M., Green, H. J., Tarnopolsky, M. A., Heigenhauser, G. J. F., Hill, R. E., and Grant, S. M. (1996). Effects of training duration on substrate turnover and oxidation during exercise. *J. Appl. Physiol.* 81, 2182–2191. doi:10.1152/jappl.1996.81.5.2182

Wood, E., Hall, K. H., and Tate, W. (2021). Role of mitochondria, oxidative stress and the response to antioxidants in myalgic encephalomyelitis/chronic fatigue syndrome: a possible approach to SARS-CoV-2 'long-haulers. *Chronic Dis. Transl. Med.* 7, 14–26. doi:10.1016/j.cdtm.2020.11.002

World Health Organization, (2023). *WHO coronavirus (COVID-19) Dashboard*. World Health Organization, Geneva, Switzerland https://covid19.who.int/.

frontiers | Frontiers in Physiology

TYPE Correction
PUBLISHED 15 March 2024
DOI 10.3389/fphys.2024.1374886

OPEN ACCESS

APPROVED BY
Frontiers Editorial Office,
Frontiers Media SA, Switzerland

*CORRESPONDENCE
Andrea Meloni,
andrea.meloni@unimi.it

RECEIVED 22 January 2024
ACCEPTED 06 March 2024
PUBLISHED 15 March 2024

CITATION
Meloni A, Codella R, Gotti D, Di Gennaro S, Luzi L and Filipas L (2024), Corrigendum: Fat oxidation rates and cardiorespiratory responses during exercise in different subject populations with post-acute sequelae of SARS-CoV-2 infection: a comparison with normative percentile values.
Front. Physiol. 15:1374886.
doi: 10.3389/fphys.2024.1374886

Corrigendum: Fat oxidation rates and cardiorespiratory responses during exercise in different subject populations with post-acute sequelae of SARS-CoV-2 infection: a comparison with normative percentile values

Andrea Meloni[1,2]*, Roberto Codella[1,2], Daniel Gotti[1], Simone Di Gennaro[3], Livio Luzi[1,2] and Luca Filipas[1,2]

[1]Department of Biomedical Sciences for Health, Università degli Studi di Milano, Milan, Italy, [2]Department of Endocrinology, Nutrition and Metabolic Diseases, IRCCS MultiMedica, Milan, Italy, [3]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics and Mother-Child Sciences, Università degli Studi di Genova, Genova, Italy

KEYWORDS

post-acute sequelae of SARS-CoV-2 infection, fat oxidation, metabolic dysfunction, cycling, exercise performance

A Corrigendum on
Fat oxidation rates and cardiorespiratory responses during exercise in different subject populations with post-acute sequelae of SARS-CoV-2 infection: a comparison with normative percentile values

by Meloni A, Codella R, Gotti D, Di Gennaro S, Luzi L and Filipas L (2023). Front. Physiol. 14: 1310319. doi: 10.3389/fphys.2023.1310319

In the published article, there was an error in the **Funding** statement. It originally stated that the authors declared no financial support was received for the research, authorship, and/or publication of this article. The correct **Funding** statement appears below.

Funding

The authors declare financial support was received for the publication of this article. This work has been supported by the Italian Ministry of Health–Ricerca Corrente–IRCCS MultiMedica.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

Publisher's note

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 12 December 2023
DOI 10.3389/fphys.2023.1186296

OPEN ACCESS

EDITED BY
Tania Gamberi,
University of Florence, Italy

REVIEWED BY
Agnieszka Zembron-Lacny,
University of Zielona Gora, Poland
Dariusz Pytel,
Medical University of South Carolina, United States
Mohamed Romdhani,
Université Paris Nanterre, France
Imed Latiri,
University of Sousse, Tunisia

*CORRESPONDENCE
Dariusz Nowak,
dariusz.nowak@umed.lodz.pl
Robert Stawski,
robert.stawski@umed.lodz.pl

RECEIVED 14 March 2023
ACCEPTED 02 November 2023
PUBLISHED 12 December 2023

CITATION
Chmielecki A, Bortnik K, Galczynski S, Kopacz K, Padula G, Jerczynska H, Stawski R and Nowak D (2023), Interleukin-4 during post-exercise recovery negatively correlates with the production of phagocyte-generated oxidants.
Front. Physiol. 14:1186296.
doi: 10.3389/fphys.2023.1186296

Interleukin-4 during post-exercise recovery negatively correlates with the production of phagocyte-generated oxidants

Adam Chmielecki[1], Krzysztof Bortnik[1], Szymon Galczynski[2], Karolina Kopacz[2], Gianluca Padula[2], Hanna Jerczynska[3], Robert Stawski[4]* and Dariusz Nowak[4]*

[1]Sports Centre, Medical University of Lodz, Łódź, Poland, [2]Academic Laboratory of Movement and Human Physical Performance "DynamoLab", Medical University of Lodz, Łódź, Poland, [3]Central Scientific Laboratory, Medical University of Lodz, Łódź, Poland, [4]Department of Clinical Physiology, Medical University of Lodz, Łódź, Poland

Exhaustive run induced a biphasic oxidative response of circulating phagocytes in 16 amateur sportsmen. The first phase involved an increment just after exercise of enhanced whole blood chemiluminescence normalized per phagocyte count, whereas in the second phase a decrement from 1 h post-exercise and ongoing till 24 h. We tested whether plasma Interleukin IL-4, IL-8, IL-10 and Tumor Necrosis Factor α concentrations change in response to exhaustive run and whether there are associations between their levels and delta resting. Moreover, IL-8 and IL-10 significantly increased immediately post-exercise and after 1 h, but later normalized. Tumor necrosis factor α rose by 1.1-times only just after exercise. However, none of these cytokines showed any correlation with the investigated chemiluminescence. Exercise did not alter plasma concentrations of IL-4. However, pre-exercise IL-4 negatively correlated with measured luminescence just after exercise ($\rho = -0.54$, $p < 0.05$), and also tended to be negatively associated with decrements of the second phase at 1 h post-exercise $\rho = -0.45$, $p = 0.08$. It is suggested that plasma IL-4, by a negative association with blood phagocytes oxidants production, could be involved in the maintenance of proper balance between oxidants and anti-oxidants during strenuous exercise and post-exercise recovery.

KEYWORDS

exhaustive exercise, interleukin-4, pro-inflammatory cytokines, phagocytes, whole blood luminescence, reactive oxygen species, ROS, granulocytes

Abbreviations: IL-4, interleukin 4; IL-6, interleukin 6; IL-8, interleukin 8; IL-10, interleukin 10; TNF-α, tumor necrosis factor alpha; VO_2, max maximal oxygen consumption; LBCL, luminol-enhanced whole blood chemiluminescence; a-rLBCL, absolute resting (spontaneous) LBCL; rLBCL, resting (spontaneous) LBCL normalized per phagocyte count; PMNs, polymorphonuclear leukocytes; ROS, reactive oxygen species; NETs, neutrophil extracellular traps; cf-nDNA, circulating cell free nuclear DNA; PMA, phorbol 12-myristate 13-acetate; RLU, relative light units; CK, creatine kinase.

1 Introduction

Strenuous exercise was reported to induce numerous immuno-metabolic responses in well-trained and untrained subjects (Nieman and Pence, 2020; Padilha et al., 2021). These include, for instance, the release of various cytokines (e.g., IL-6, IL-8, IL-10), an increase in intramuscular activities of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase), changes in the number and activity of circulating neutrophils, monocytes, dendritic cells, T cells, and natural killer cells, as well as mitochondrial biogenesis and alterations in gut microbiome (Walsh et al., 2011; Nieman and Pence, 2020; Padilha et al., 2021). Moreover, numerous circulating oxylipins generated during exercise may alter the function of immune system cells (Nieman and Pence, 2020). Although these responses, induced by a single bout of exercise, are transient, properly selected, regular lifelong exercise can delay aging-related immunosenescence (Padilha et al., 2021). On the other hand, prolonged training overload may induce a persistent inflammatory response and oxidative stress, which are contributors to the development of overtraining syndrome (Tanskanen et al., 2010; Cheng et al., 2020). Recently, we found that exhaustive treadmill exercise at a speed corresponding to 70% of personal VO_2 max caused an increase in resting (spontaneous) production of reactive oxygen species (ROS) by circulating polymorphonuclear leukocytes (PMNs), evaluated by luminol enhanced whole blood chemiluminescence (LBCL) (Chmielecki et al., 2022). This change was observed for both absolute resting chemiluminescence (a-rLBCL—light emission generated by 3 µL of the assayed blood sample) and for chemiluminescence calculated per 103 phagocytes present in the assayed blood sample (rLBCL). However, exercise-induced increase in ROS production was transient and absolute resting chemiluminescence at 1 h post-exercise did not differ from the pre-exercise baseline values. Moreover, resting chemiluminescence s after 1 h from the end of the bout was about 2-times lower than pre-exercise values, and this inhibition persisted till 24 h post-exercise (Chmielecki et al., 2022). Analogous observation, in similar conditions has reported also Yamada et al. (2000); Quindry et al. (2003). We supposed that this increase immediately after exercise in a resting chemiluminescence and rLBCL was related to exercise-induced NETosis (Chmielecki et al., 2022), which is a program for formation of neutrophil extracellular traps (NETs). It is a unique form of cell death that is characterized by the release of decondensed chromatin and other granular contents to the extracellular space. Noteworhty NETosis is offenly accompanied by ROS production (Vorobjeva and Chernyak, 2020) and had similar kinetics (Velders et al., 2014). We also postulated that biphasic changes of resting chemiluminescence (rise just after exercise and then significant suppression) could be the consequence of a parallel increase in circulating anti-inflammatory IL-10 (Chmielecki et al., 2022). IL-10 was reported to rise in response to exercise (Cabral-Santos et al., 2015; Cabral-Santos et al., 2019) and *in vitro* inhibited respiratory burst of PMNs after stimulation with various agonists (Chaves et al., 1996; Capsoni et al., 1997; Dang et al., 2006). Nevertheless, PMNs during and after the exhaustive treadmill run are exposed to a variety of pro- and anti-inflammatory cytokines as TNF-α and IL-8, IL-4, for example,. The first two previously listed are able to prime and stimulate ROS generation from PMNs (You et al., 1991; Baggiolini and Clark-Lewis, 1992; Gougerot-Podicalo et al., 1996; Decleva et al., 2002), while IL-4 was reported to inhibit PMA (phorbol 12-myristate 13-acetate)-induced neutrophil extracellular traps (NETs) formation *in vitro* (Impellizzieri et al., 2019). Having stored plasma specimens obtained from our previous study (Chmielecki et al., 2022), we decided to measure concentrations of these four cytokines (IL-10, TNF-α, IL-8 and IL-4). Hence we hypothesized: (1)—there is an effect of exhaustive exercise on circulating cytokins within 24 h from the end of exercise, and (2)—there is possible associations between circulating cytokins and exercise-induced changes in ROS production by blood PMNs.

2 Materials and methods

2.1 Studied group

The studied group of 18 average trained non-smoking, healthy men (10 soccer players and 8 powerlifters). The inclusion/exclusion criteria and the study protocol were the same as in our previous report (Chmielecki et al., 2022). Volunteers had mean age 22 ± 2 years, mean body mass 80 ± 12 kg, mean body mass index 24.3 ± 2.6 kg/m^2, and a mean maximal oxygen consumption 49 ± 5 mL/kg.min^{-1} (Supplementary Tables S1, S2). There were no significant differences between soccer players and powerlifters in respect to the afore-mentioned variables probably due to the short training experience. Exclusion criteria were as follows: presence of contraindications to exhaustive treadmill exercise, presence of any injuries which may limit exercise performance, current cigarette smoking, alcohol and illicit drug abuse, use of any vitamins, food supplements, antioxidants or any systemic pharmacological treatment or history of acute infectious or inflammatory diseases within 3 months prior to the study.

2.2 Study protocol

Briefly, the study had a cross over design and consisted of four visits on the 0th, 7th, 14th, and 28th day of observation (Chmielecki et al., 2022) (Table 1). The first two visits (0th and 7th) were focused on volunteers eligibility criteria, obtaining informed consent, collecting demographic data, exclusion of contraindications to exercise stress, assessment of VO_2max, and randomization with assignment to arm 1 or 2. During the third visit (14th), half of the randomly selected participants (arm 1, $n = 9$) performed a treadmill run to volitional exhaustion at a speed corresponding to 70% of their personal VO_2max. Subjects were only allowed to drink mineral water at will during the treadmill run, and pre- and post-exercise heart rate, arterial blood pressure, and body mass were measured. Venous blood samples (15 mL) were collected six times into Becton Dickinson vacutainer tubes (with EDTA for blood cell count, for resting chemiluminescence and for IL-4, IL-8, IL-10 and TNF-α evaluation) with gel and clot activator for blood chemistry and with sodium oxalate and potassium fluoride for lactate measurement before, just after, and at 1, 3, 5 and 24 h after the exhaustive exercise. Blood chemistry and lactate were determined only in pre- and immediately post-exercise blood samples. EDTA anticoagulated

TABLE 1 Study design and summary of protocol procedures.

Visit number	Day of the study, time	Procedures performed during the visit	
1	0th, 9 a.m.–2 p.m.	Volunteers enrollment (eligibility criteria - inclusion/exclusion criteria), informed consent, instructing the volunteer about all study procedures	
2	7th, 9:30 a.m.	Medical examination, resting ECG, spirometry, arterial blood pressure, treadmill VO_2max assessment, randomization	
3	14th, 9:30 a.m.	Medical examination, resting ECG, arterial blood pressure, blood collections—before, just after, and at 1, 3, 5 and 24 h post-exercise	
		Arm 1	Arm 2
		Treadmill (constant inclination of 1.5%) run to volitional exhaustion at a speed corresponding to 70% of personal VO_2max	1 h resting instead of treadmill run
4	28th, 9:30 a.m.	Medical examination, resting ECG, arterial blood pressure, blood collections—before, just after, and at 1, 3, 5 and 24 h post-exercise	
		1 h resting instead of treadmill run	Treadmill (constant inclination of 1.5%) run to volitional exhaustion at speed corresponding to 70% of personal VO_2 max

a.m.—Ante Meridiem, ECG-electrocardiography, VO_2max–maximal oxygen consumption.

blood samples were centrifuged for 15 min (1000 × g, 4°C) and the obtained plasma was stored at −80°C (up to 5 months), until cytokine measurement. Volunteers allocated to arm 2 (n = 9) underwent the same procedures but without the exhaustive treadmill exercise. At the 4th visit (day 28th), subjects from arm 2 performed the exhaustive treadmill run while those from arm 1 were without any physical effort (Table 1). Volunteers nutrition, training load restrictions during this 28 days study, and ambient conditions during the exhaustive treadmill run have been described in detail in our previous work (Chmielecki et al., 2022). This study was conducted according to the Declaration of Helsinki, the protocol was reviewed and approved by The Medical University of Lodz Ethics Committee (RNN/118/17/KE), and all volunteers provided a written informed consent.

Volitional exhaustion was defined as the volunteers' inability to maintain the required exercise intensity or their will to stop the treadmill run, despite a strong encouragement to continue by the testing staff. When symptoms of volitional exhaustion appeared, the exercise test was terminated. Mean run distance to exhaustion was 13.9 ± 5.1 km, run time 76 ± 26 min, heart rate at the end of the run 167 ± 12 beats/min, representing 84% ± 7% of maximal heart rate, and 1.2 ± 0.5 kg loss of body mass.

2.3 Description of VO_2 max and execution of exhaustive treadmill run

The exhausting treadmill run and the VO_2 max measurement (continuous incremental maximal exercise test) were carried out in accordance with the same protocol, using the same technical tools, and under the same circumstances as previously mentioned (Chmielecki et al., 2022). Briefly, volunteers began running on a treadmill (continuous incline of 1.5%) at a pace of 7 km/h; this speed was increased by 1.5 km/h every 3 minutes until voluntary exhaustion. To estimate VO_2 max, three conditions required to be met: (A) a plateau in oxygen consumption despite an increase in running speed; (B) a respiratory exchange ratio more than 1.10; and (C) a peak heart rate greater than 90% of the age-predicted maximum (220—age). The strenuous exercise was a treadmill run at a speed equal to 70% of the body's measured maximum heart rate.

2.4 Measurement of the luminol-enhanced whole blood chemiluminescence

Resting luminol enhanced whole blood chemiluminescence, as a measure of *ex vivo* spontaneous ROS production by circulating phagocytes, was carried out according to the protocol described by Kukovetz et al. (1997) with our modifications (Bialasiewicz et al., 2014; Bialasiewicz et al., 2018) described in detail in our previous study (Chmielecki et al., 2022). The luminol solution was prepared by dissolving 25 mg luminol in 90 mL (0.1 mol/L) Na2HPO4, then the pH was adjusted to 7.4 with 1 mol/L HCl, and the volume was made up to 100 mL with distilled water. After immediate filtration (0.2 mm Millipore filter), it was stored at 4°C in the dark for no longer than 2 weeks. The mixture solution was prepared just before the blood chemiluminescence assay by adding 1 mL of Ringer's solution (155.7 mmol/L NaCl, 5.36 mmol/L KCl, 1.78 mmol/L NaHCO3, pH = 7.4), 5 mL of luminol solution, and 0.2 mL of 277.5 mmol/L glucose solution to 3.6 mL distilled water. Briefly, blood samples were initially diluted with a mixture solution (30 µL of blood added to 1000 µL of mixture solution). Then, 103 µL of diluted blood was added to a tube (Lumi Vial Tube, 5 mL, 12 × 75 mm, Berthold Technologies, Bad Wildbad, Germany) containing 797 µL of mixture solution (which resulted in a final blood dilution of 300 times), placed in a multitube luminometer (AutoLumat Plus LB 953, Berthold, Germany) and incubated for 15 min at 37°C in the dark. Then, 100 µL of the control solution (0.9% NaCl with addition of 1% v/v DMSO) for measurement of absolute resting chemiluminescence was injected by an automatic dispenser, and, after 7 s, the total light emission was automatically measured for 120 s. Individual results (given in relative light units—RLU) were obtained as the means of triplicate experiments. To exclude the possible fluctuations of the background noise signal and its effect on chemiluminescence, the light emission from samples containing only 900 µL of mixture solution was measured before and after each series of six studied samples, then the mean background noise signal was subtracted from the corresponding individual results of absolute resting chemiluminescence. Results of resting chemiluminescence were expressed in two ways: (A) as absolute light emission generated by 3 µL of assayed blood sample absolute resting chemiluminescence, and (B) as light emission per 10^3 phagocytes

(granulocytes and monocytes) present in the assayed blood sample resting chemiluminescence.

2.5 Measurement of IL-4, IL-8, IL-10 and TNF-α in plasma samples

Concentrations of IL-4, IL-8, IL-10 and TNF-α were simultaneously measured in each plasma sample using a multiplex kit based on the Luminex xMAP technology (Human High Sensitivity Cytokine A Assay; Cat # FCSTM09, R&D Systems, United States). All procedures were strictly executed according to the manufacturer instruction (Magnetic Luminex Performance Assay, www.RnDSystems.com). The results are presented as the mean of two repeats whereas the intra assay CV is parameter automatically analyzed by algorithm attached to the equipment thus results with CV higher than 10% were reapeted. Briefly, four types of magnetic beads pre-coated with antibodies specific for each afore-mentioned analyte (25 µL of diluted Microparticle Cocktail) were added to the wells containing 100 µL of standards, plasma samples or controls, and incubated for 3 h. During this incubation step, analytes of interest from the standard and plasma samples were captured by the appropriate beads. Then, the magnetic beads were washed three times with 100 µL of Wash Buffer and a cocktail of biotinylated antibodies specific for each cytokine (50 µL of diluted Biotin-Antibody Cocktail) was added and incubated for 1 h. Afterwards, all unbound biotinylated antibodies were washed away and 50 µL of streptavidin-phycoerythrin (PE) conjugate was added to detect biotinylated antibodies on the surface of each bead (30 min incubation). At the end, the unbound streptavidin-PE conjugate was removed, the magnetic beads were resuspended in 100 µL of Wash Buffer, and a fluorescence signal proportional to the amount of analyte bound was read on a Luminex® MAGPIX® analyzer (Luminex, United States). All incubations were performed at room temperature and on a shaker at 800 rpm. In the analyzer, the magnetic beads captured in a monolayer were illuminated with two spectrally different diodes (LEDs). One (red) to excite the dyes inside the beads and identify each cytokine, the other (green) to excite PE and measure the amount of analyte bound to the bead. All results were analyzed with Belysa software 1.1.0 (Millipore Sigma, Burlington, MA, United States) and the protein concentration (pg/mL) was determined by interpolation from the standard curve against a five parameter logistic curve. The standard curve range for IL-4, IL-8, IL-10 and TNF-α were 6.6–6 745 pg/mL, 0.8–3 370 pg/mL, 0.5–2 075 pg/mL and 0.8–3 185 pg/mL, respectively. Due to the accidental loss of two sets of plasma samples, the afore-mentioned evaluations were performed for 16 male athletes. Thus, all results related to cytokines including correlations were obtained from a group of 16 subjects.

2.6 Other analyses

Blood chemistry (creatine kinase–CK, aspartate amino-transferase, alanine aminotransferase, lactate, urea, creatinine) was determined in the Diagnostic Laboratory of the Central Clinical Hospital of the Medical University in Lodz. Blood cell count was measured with a Micros Analyzer OT 45 (ABX, Montpellier, France). VO_2 max was determined by a continuous incremental maximal exercise treadmill test (constant inclination of 1.5%, initial speed of 7 km/h increased every 3 min by 1.5 km/h until volitional exhaustion) as previously described (Stawski et al., 2017; Stawski et al., 2019). Three criteria had to be met to determine the VO_2 max: (A) a plateau in the O2 consumption despite an increase in running velocity, (B) a respiratory exchange ratio higher than 1.10, and (C) a peak heart rate higher than 90% of the age-predicted maximum (220-age) (Tanaka et al., 2001).

2.7 Statistical analysis

Results were expressed as means (SD) and medians (interquartile range). Analysis of variance (ANOVA) for repeated observations (parametric test) or Friedman's ANOVA (nonparametric test) was applied for the assessment of changes in variables over time (pre-exercise, just after, and at 1, 3, 5 and 24 h post-exercise) depending on the data distribution tested with Shapiro-Wilk's W test. When ANOVA was statistically significant, *post hoc* analyses were done with Scheffe's test or *post hoc* analysis for Friedman's ANOVA (multiple comparisons at two different time-points). Individual changes in absolute resting chemiluminescence and resting chemiluminescence from the baseline at given time-point after the exhaustive run (Δa-rLBCL, ΔrLBCL) were calculated by subtracting the pre-exercise value from the appropriate post-exercise value. The relationships between individual changes (increase or decrease) in rLBCL or a-rLBCL and corresponding concentrations of circulating cytokines (IL-4, IL-8, IL-10 and TNF-α) were analyzed with Spearman's rank correlation coefficient test (Spearman's ρ). Other associations (e.g., between cytokine concentrations and parameters of treadmill run, markers of muscle damage and metabolic response to exercise) were determined in the same way. A p value < 0.05 was considered significant. Despite the fact that number of participants was low, our analysis confirmed that the number of participants was sufficient for all analytes besides IL-10. Thus, individual data have been added in the Supplementary File. All calculations and analyses were performed with Dell Statistica (data analysis software system), version 13 (Dell Inc. 2016).

3 Results

3.1 Muscle damage and metabolic response to exhaustive exercise

This part has been accurately described in our previous study (Chmielecki et al., 2022). Among the six studied markers, aspartate aminotransferase, alanine aminotransferase, creatine kinase, lactate, creatinine and urea, the last four increased significantly after the exhaustive run. The exercise-induced increase in CK, lactate, creatinine and urea were from 180 ± 59 to 233 ± 56 U/L, from 1.7 ± 0.3 to 3.0 ± 1.1 mmol/L, from 88 ± 9 to 106 ± 16 µmol/L and from 5.7 ± 1.1 to 6.5 ± 1.1 mmol/L ($p < 0.05$), respectively. The number of phagocytes (neutrophils plus monocytes) (×103/µL) in the peripheral blood increased from a pre-exercise baseline value of 3.25 ± 1.52 to 5.35 ± 2.49 just after exercise; 6.57 ± 3.39; 8.03 ±

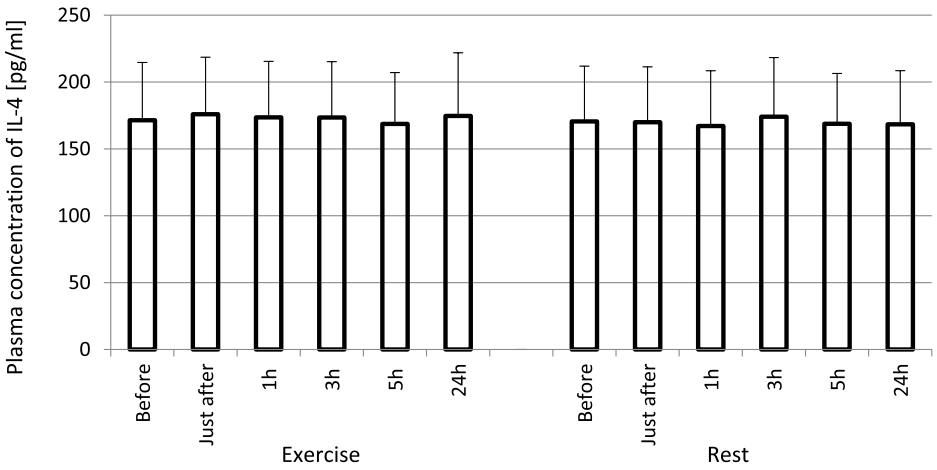


FIGURE 1
No effect of exhaustive treadmill run on plasma concentrations of IL-4 in amateur athletes. Exercise—subjects performed a treadmill run to volitional exhaustion at a speed corresponding to 70% personal VO_2 max and blood was collected before, just after, and at 1, 3, 5, and 24 h post-exercise. Rest—control, the same protocol but without any physical activity. Results are expressed as Mean (SD).

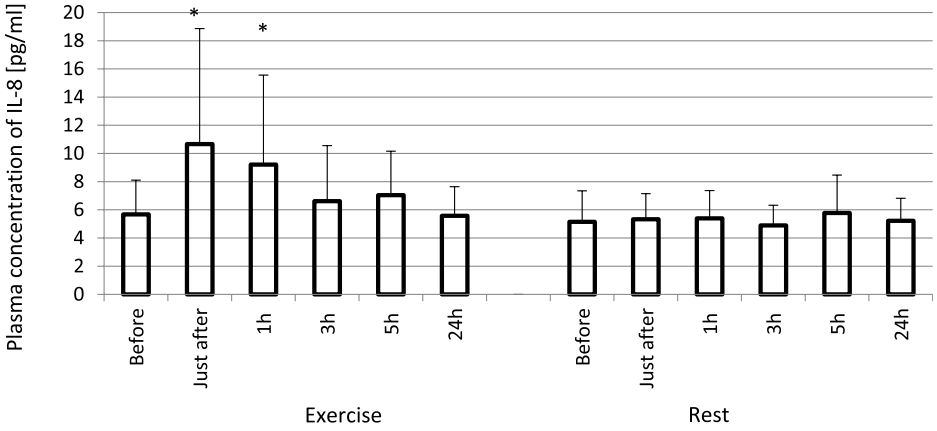


FIGURE 2
Exhaustive exercise-induced increase in plasma concentration of IL-8 in amateur athletes. *—vs. pre-exercise (before) value, $p < 0.05$. Exercise subjects performed a treadmill run to volitional exhaustion at a speed corresponding to 70% personal VO_2 max and blood was collected before, just after, and at 1, 3, 5, and 24 h post-exercise. Rest—control, the same protocol but without any physical activity. Results are expressed as Mean (SD).

3.78 and 6.45 ± 3.31 at 1, 3, and 5 h post-exercise, respectively, and returned to baseline at 24 h post-exercise (3.12 ± 1.34).

3.2 Effect of exercise on plasma concentrations of IL-4, IL-8, IL-10 and TNF-α

The exhaustive treadmill run had no effect on circulating concentrations of IL-4 (Figure 1). The post-exercise concentrations ranged from 169 ± 38 (172; 49) pg/mL at 5 h post-exercise to 176 ± 43 (173; 51) pg/mL just after the exercise, and did not differ from pre-exercise ones [171 ± 43(175; 55) pg/mL] as well as to those observed when the exercise was replaced by 1 h resting period (Figure 1). The remaining three cytokines IL-8, IL-10 and TNF-α raised significantly in response to exhaustive run (Figures 2, 3, 4). Mean plasma concentrations of IL-8 observed just after [10.7 ± 8.2 (7.6; 4.1) pg/mL] and at 1 h post-exercise [9.2 ± 6.3 (7.1; 2.9) pg/mL] were 1.9- and 1.6-times higher than those before the bout [5.7 ± 2.4 (5.0 ± 1.8) pg/mL]. Then, the IL-8 level gradually decreased at 24 h post-exercise to a value close to baseline (Figure 2). Similarly behaved IL-10, its concentrations just after [4.7 ± 3.0 (4.5; 3.6) pg/mL] and at 1 h post-exercise [4.4 ± 2.2 (4.0; 3.9) pg/mL] were almost 2-times and 1.8- times higher ($p < 0.05$) than the pre-exercise baseline value [2.4 ± 0.9 (2.5; 1.8) pg/mL], with a following normalization already at 3 h post-exercise (Figure 3). Moderate increase in plasma concentration of TNF-α was noted only just after exercise in comparison to pre-exercise baseline: 17.3 ± 3.1 (16.8 ± 3.6) pg/mL vs. 15.5 ± 3.4 (15.5; 3.9) pg/mL, $p < 0.05$ (Figure 4). No statistically significant IL-8, IL-10 and TNF-α

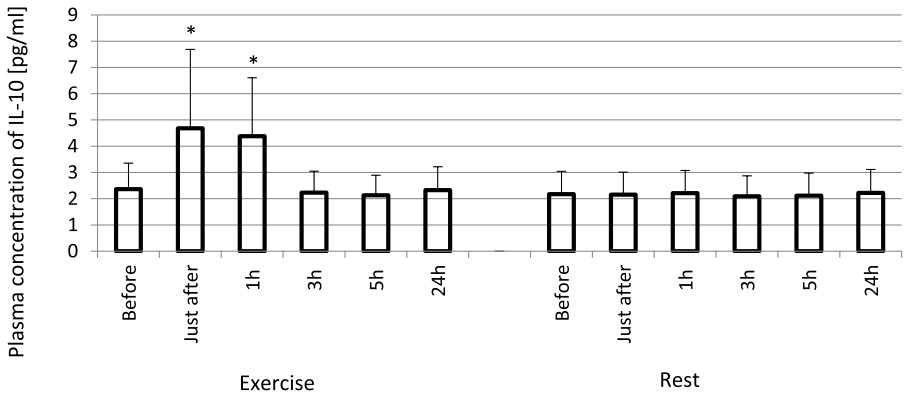


FIGURE 3
Exhaustive exercise-induced increase in plasma concentration of IL-10 in amateur athletes. *—vs. pre-exercise (before) value, $p < 0.05$. Exercise subjects performed a treadmill run to volitional exhaustion at a speed corresponding to 70% personal VO_2 max and blood was collected before, just after, and at 1, 3, 5, and 24 h post-exercise. Rest—control, the same protocol but without any physical activity. Results are expressed as Mean (SD).

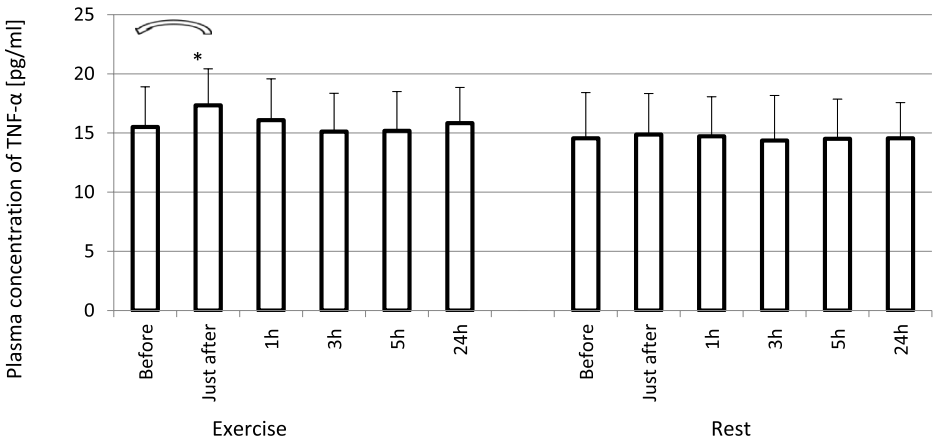


FIGURE 4
Changes of circulating concentrations of TNF-α in response to an exhaustive treadmill run. *—vs. pre-exercise (before) value, $p < 0.05$. Exercise subjects performed a treadmill run to volitional exhaustion at a speed corresponding to 70% personal VO_2 max and blood was collected before, just after, and at 1, 3, 5, and 24 h post-exercise. Rest—control, the same protocol but without any physical activity. Results are expressed as Mean (SD).

fluctuations were observed in the resting paticipants, i.e., the group without any physical activity (Figures 2, 3, 4) (Supplementary Tables S3–S6).

3.3 Effect of exercise on absolute rLBCL (a-rLBCL) and rLBCL expressed as light emission per 10^3 phagocytes (normalized per phagocyte count)

In order to analyze the associations between spontaneous ROS production by circulating phagocytes and plasma concentration of selected cytokines, we calculated rLBCL and a-rLBCL changes from baseline (ΔrLBCL, Δa-rLBCL) over 24 h of post-exercise observations. ΔrLBCL reached 239 ± 398 (193; 495) RLU just after the exercise and −251 ± 467 (−131; 277) RLU, −217 ± 452 (−105; 259) RLU, −216 ± 480 (−118; 196) RLU, −256 ± 432 (−111; 374) RLU at 1, 3, 5 and 24 h post-exercise, respectively (Figure 5), depicting significant enhancement (first time-point) or inhibition (the remaining time-points) of ROS production by exhaustive exercise. In control experiments (rest), ΔrLBCL ranged from −46 ± 222 RLU to −117 ± 237 RLU at 24 h and 5 h post-exercise, illustrating no significant alterations of ROS production in the absence of exhaustive exercise. Δa-rLBCL was 6907 ± 8167 (4356; 2535) RLU and −2673 ± 3965 (−1085; 4154) RLU just after and at 24 h post-exercise representing significant enhancement and inhibition in response to exercise, respectively (Figure 6).

3.4 Correlations between selected parameters of exhaustive run and increased post-exercise cytokines

Increased concentrations of IL-8 (just after exercise and at 1 h post -exercise) and TNF-α (just after exercise) did not correlate with

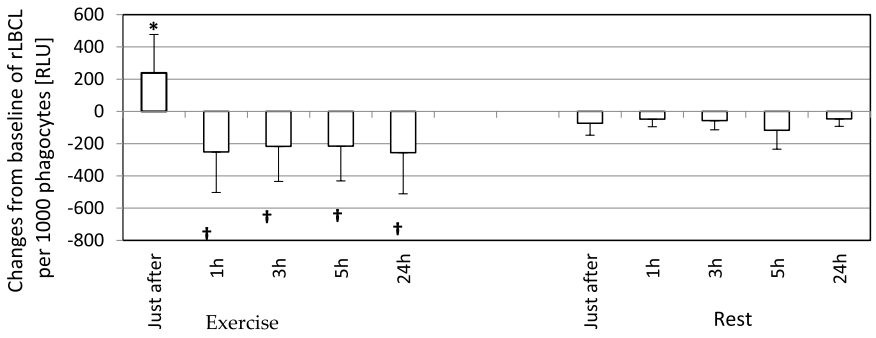


FIGURE 5
Changes from baseline of rLBCL (light emission per 10^3 phagocytes) observed just after, and at 1, 3, 5 and 24 h post-exercise. As a baseline served pre-exercise rLBCL. Exercise—eighteen athletes performed a treadmill run to volitional exhaustion at a speed corresponding to 70% personal VO_2 max and blood was collected before, just after, and at 1, 3, 5, and 24 h post-exercise. Rest - control, the same protocol but without any physical activity. Results are expressed as Mean (SD). *—significant enhancement, †—significant inhibition. The response of rLBCL to an exhaustive run is biphasic: a first phase - increase just after excise; a second phase—decrease at 1 h post-exercise ongoing until 24 h post-exercise.

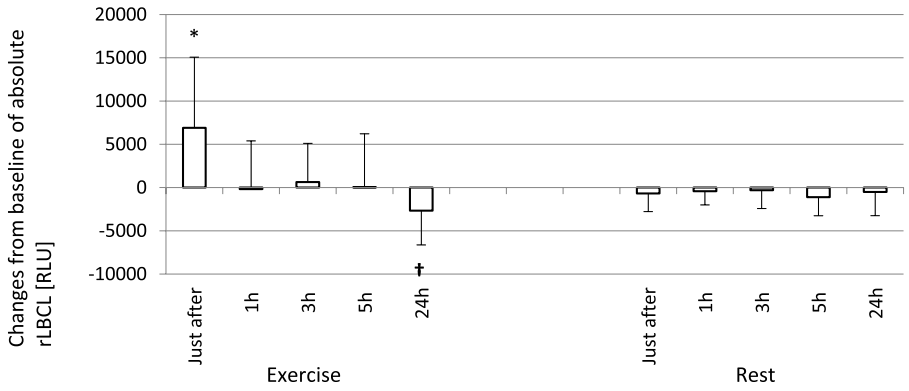


FIGURE 6
Changes from baseline of absolute rLBCL (a-rLBCL - light emission generated by phagocytes present in 3 µL of assayed blood sample) were observed just after and 1, 3, 5 and 24 h post-exercise. As a baseline served pre-exercise a-rLBCL. Other details as for Figure 5. Results are expressed as Mean (SD). *—significant enhancement, †—significant inhibition. The response of a-rLBCL to an exhaustive run is also biphasic: a first phase - increase just after the exercise, and a second phase - decrease at 24 h post-exercise are separated by a plateau (time points 1, 3, and 5 h post-exercise).

the run distance, run time, heart rate at the end of run and loss of body mass. However, concentrations of IL-10 in blood specimens collected just after the exercise correlated significantly ($p < 0,05$) with the run distance ($\rho = 0.62$) (Figure 7), run time ($\rho = 0.57$), and loss of body mass ($\rho = 0.58$). Similarly, there were significant positive associations between elevated plasma levels of IL-10 at 1 h post-exercise with run distance and run time (Table 2).

3.5 Correlations between exercise-induced increment in luminol enhanced whole blood chemiluminescence and cytokine concentrations

Exhaustive treadmill run increased a-rLBCL and rLBCL of blood samples collected just after the bout. We tested whether pre-exercise or just after the exercise plasma concentrations of four monitored cytokines (IL-4, IL-8, IL-10 and TNF-α) correlated with Δa-rLBCL or ΔrLBCL. TNF-α and IL-8 did not associate either with Δa-rLBCL or with ΔrLBCL (Table 3). There was also no significant association between pre-exercise and just-after-exercise plasma IL-10 levels and Δa-rLBCL. On the other hand, there was a negative correlation between pre-exercise IL-10 and ΔrLBCL ($\rho = -0.46$) that reached borderline significance ($p = 0.075$). Pre-exercise circulating IL-4 negatively correlated ($p < 0.05$) with Δa-rLBCL ($\rho = -0.51$) and ΔrLBCL ($\rho = -0.54$) (Figure 8).

3.6 Correlations between post-exercise decrements (-Δ) in spontaneous luminol enhanced whole blood chemiluminescence and cytokine concentrations

Blood samples collected from male athletes revealed decreased rLBCL at 1, 3, 5, and 24 h post-exercise in comparison to pre-exercise baseline. We analyzed whether circulating concentrations of

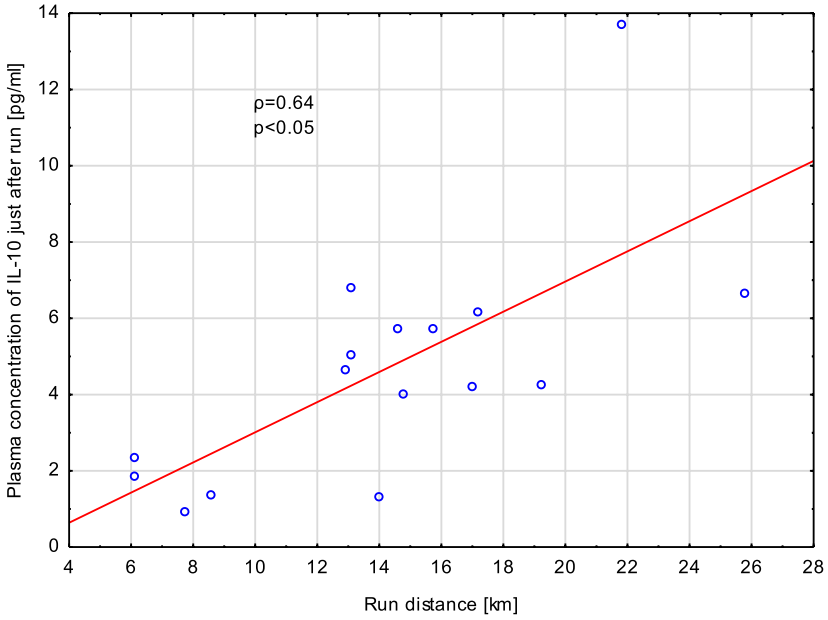


FIGURE 7
Correlation (Spearman's ρ) between plasma concentration of IL-10 in samples collected just after exhaustive exercise and run distance. Athletes performed a treadmill run to volitional exhaustion at a speed corresponding to 70% personal VO_2 max.

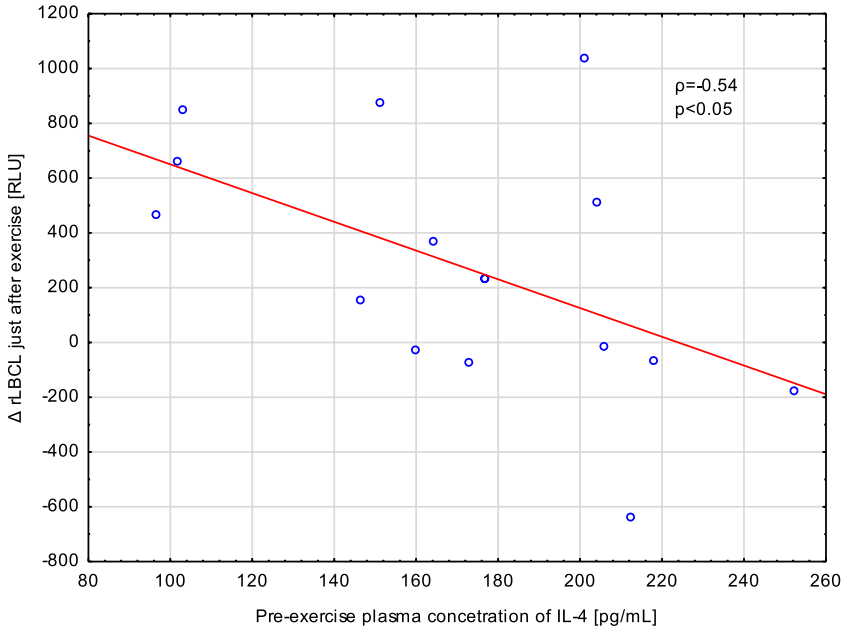


FIGURE 8
Negative correlation (Spearman's ρ) between the increment in spontaneous luminol enhanced blood chemiluminescence per 10^3 phagocytes (ΔrLBCL) just after exercise and pre-exercise plasma concentration of IL-4 in male athletes who performed a treadmill run to volitional exhaustion at a speed corresponding to 70% personal VO_2 max.

cytokines (IL-4, IL-8, IL-10 and TNF-α) associated with these decrements in rLBCL. Delat rLBCL (ΔrLBCL) at 1 h post-exercise was correlated with pre-, just-after and 1 h post-exercise concentrations of a given cytokine while ΔrLBCL at 24 h post-exercise was correlated with six sets of 16 individual cytokine levels from 6 time-points what gives in total 96 pairs. For all analyzed pairs (n = 96) of ΔrLBCL and cytokine concentration, no significant correlations were found. However, it should be pointed out that for

TABLE 2 Correlations (Spearman's ρ) between increased post-exercise concentrations of plasma IL-8, IL-10, TNF-α and selected parameters of exhaustive treadmill run.

Parameter	Concentrations of circulating cytokines †				
	IL-8		IL-10		TNF-α
	Just after exercise	1 h post-exercise	Just after exercise	1 h post-exercise	Just after exercise
Run distance	0.35	0.18	0.62*	0.63*	0.07
Run time	0.30	0.11	0.57*	0.57*	0.09
Heart rate at the end of run	0.22	0.43	0.19	0.30	−0.32
Loss of body mass‡	0.45	−0.04	0.58*	0.45	−0.04

†—correlations were calculated only for time-points at which a significant rise of IL-8, IL-10, and TNF-α, was noted.
‡—due to water loss with sweat and evaporation from the surface of airways. Athletes performed a treadmill run to volitional exhaustion at a speed corresponding to 70% personal VO_2 max. Blood was collected before, just after, and at 1, 3, 5, and 24 h post-exercise. *-p<0.05.

TABLE 3 Correlations (Spearman's ρ) between the increment (+Δ) of resting blood chemiluminescence observed just after the exhaustive exercise and plasma cytokine concentrations (IL-4, IL-8, IL-10 and TNF-α).

Plasma cytokine concentrations [pg/mL]	Increment (+Δ) in blood chemiluminescence [RLU] just after the exhaustive treadmill run	
	Δa-rLBCL	ΔrLBCL
IL-4 pre-exercise	−0.51*	−0.54*
IL-4 just after exercise	−0.40	−0.44 ‡
IL-8 pre-exercise	0.07	−0.06
IL-8 just after exercise	0.19	0.06
IL-10 pre-exercise	−0.24	−0.46 †
IL-10 just after exercise	−0.26	−0.31
TNF-α pre-exercise	0.05	0.11
TNF-α just after exercise	0.13	0.23

RLU, relative light units; a-rLBCL, absolute resting luminol enhanced whole blood chemiluminescence; rLBCL, resting luminol enhanced whole blood chemiluminescence per 10^3 phagocytes, Δa-rLBCL = (a-rLBCL just after exercise)—(pre-exercise a-rLBCL), ΔrLBCL = (rLBCL, just after exercise)—(pre-exercise rLBCL). Blood was collected before (pre-exercise), just after, and at 1, 3, 5, and 24 h post-exercise. *-p<0.05, borderline significance—‡—p = 0.099, †—p = 0.075.

the pair ΔrLBCL and IL-4 (n = 16) the Spearman's ρ was always negative and ranged from −0.45 to −0.21. Correlations between ΔrLBCL at 1 h post-exercise and pre- and just-after-exercise IL-4 reached −0.45 (p = 0.08) and −0.43 (p = 0.09), respectively (Figure 9).

4 Discussion

Exhaustive treadmill run resulted in a transient increase in plasma concentrations of three out of four monitored cytokines in amateur sportsmen. Thus, while interleukin IL-8, IL-10 and TNF were increased, IL-4 remained unafected thoughout the 24 h obsevation period. Both IL-8 and IL-10 were elevated at least up to 3 h post-exercise, whereas TNF-α increase was less pronounced and noted only just after the exercise. Only elevated concentrations (just after and at 1 h post-exercise) of IL-10 correlated significantly with exercise bout parameters such as run distance, run time and loss of body mass due to sweat and water evaporation. On the other hand, the increase in IL-8 and TNF-α in response to exercise could be a secondary phenomenon to various immuno-metabolic process activated by exhaustive run. Resting skeletal muscles contain mRNA for all the afore-mentioned cytokines (Peake et al., 2015). Circulating IL-10 and skeletal muscle IL-10 mRNA rose after exercise, while no expression of muscle IL-10 was found (Peake et al., 2015). This indicates that skeletal muscles are not a source of post-exercise increase in plasma IL-10. However, the distinct correlation between increased concentrations of IL-10 and the load of the exhaustive run observed in our study as well as reported by other authors (Cabral-Santos et al., 2019; Suzuki, 2019) suggests that skeletal muscle can generate signals inducing the secretion of IL-10 from other tissues or cells (e.g., Th2 cells) (Kakanis et al., 2014). Furhermore, other cells besides muscle fibers present in skeletal muscles such as macrophages, endothelial cells, satellite cells, fibroblast or pericytes could be involved in the post-exercise IL-10 elevation (Peake et al., 2015). Although TNF-α expression

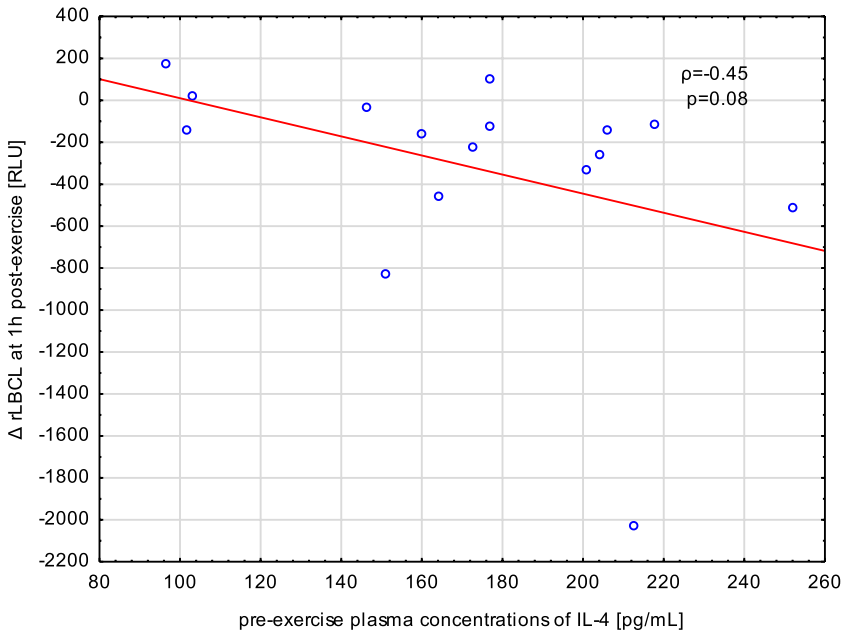


FIGURE 9
Negative correlation (Spearman's ρ) between the decrement in spontaneous luminol enhanced whole blood chemiluminescence per 10^3 phagocytes (ΔrLBCL) at 1 h post-exercise, and pre-exercise plasma concentration of IL-4 in male athletes who performed a treadmill run to volitional exhaustion at a speed corresponding to 70% personal VO_2 max.

markedly rose in muscles during exercise (Peake et al., 2015), the post-exercise circulating concentrations increased moderately and very transiently. Exercise enhanced expression of IL-8 and IL-10 mRNA in leukocytes, while spontaneous release of these cytokines from leukocytes was decreased (Peake et al., 2015), despite their increase in plasma. Therefore, further studies are necessary to precisely describe mechanisms and sources of exercise-induced changes in plasma cytokine concentrations.

In parallel to transient increase in plasma concentrations of IL-10 and TNF-α, samples of blood collected just after the exercise revealed increased rLBCL that at 1 h post-exercise and later became suppressed in comparison to pre-exercise rLBCL. Surprisingly, there were no significant correlations between ΔrLBCL and plasma concentrations of IL-8, IL-10 and TNF-α just after the exercise. Besides, pre-exercise and just-after-bout concentrations of IL-4 negatively correlated with just-after-exercise ΔrLBCL. It is supposed that changes of PMNs activity including increase in ROS production (Chmielecki et al., 2022), increase in cell-free DNA (Breitbach et al., 2012; Stawski et al., 2017), H3 histone (Stawski et al., 2021) and granular enzymes (e.g., myeloperoxidase, elastase) (Breitbach et al., 2012) in response to vigorous exercise are the consequences of increased NETs formation (Breitbach et al., 2012; Breitbach et al., 2014; Beiter et al., 2015). Moreover, recent studies showed that released cell free DNA in response to exercise almost exclusively originates from neutrophils (Neuberger et al., 2022). IL-4 was reported to inhibit NETs formation *in vitro* by imparing migration of neutrofils, by desensitizing *CXCL8*-mediated chemotaxis (Egholm et al., 2019; Impellizzieri et al., 2019), therefore, the afore-mentioned negative correlations support the concept about exercise-induced NETs formation.

4.1 Plausible mechanism of negative correlations between plasma pre-exercise IL-4 and Δ-rLBCL

Interleukin 4 (IL-4) is an important regulator of humoral and adaptive immunity. Numerous cells have receptors for IL-4 and can be activated, stimulated for proliferation or differentiation (e.g., B cells, T cells), by this cytokine. There are also receptors for IL-4 on the plasma membrane of human neutrophils of healthy subjects (Impellizzieri et al., 2019). The binding of IL-4 to these receptors induces their dimerization, activation of receptor-associated Janus kinases, and then leads to the phosphorylation and activation of signal transducer and activator of transcription proteins (Egholm et al., 2019). These phosphorylated proteins undergo dimerization and, after translocation to the nucleus, initiate transcription of target genes. In general, the effect of IL-4 on human PMNs is inhibitory, being one of the mechanisms protecting tissues from excessive damage in the course of immunological response (Egholm et al., 2019; Impellizzieri et al., 2019; Heeb et al., 2020). Preincubation of human PMNs with IL-4 at concentration of 150 ng/mL for 6 h inhibited NETs formation induced by 100 nmoles/L PMA (Impellizzieri et al., 2019). Moreover, these so treated PMNs had lower expression of chemokine receptors (CXCR1, CXCR2) and revealed lower chemotactic response to chemokine CXCL8 (Impellizzieri et al., 2019). Although the IL-4 concentration used in the afore-mentioned experiment was many times higher than that present in the serum of healthy subjects (Kleiner et al., 2013), this cannot exclude the possibility of such an effect of IL-4 *in vivo*. PMA is the strongest and most effective inducer of NETs formation (Hoppenbrouwers et al., 2017), but it is not a physiological activator. Isolation of PMNs can alter their responsiveness to

various mediators including IL-4, and so high concentrations must be used in in vitro experiments. However, to our knowledge, no studies focused on NETs inhibition by various IL-4 concentrations have been executed so far. *In vivo*, circulating neutrophils are continuously exposed to IL-4, thus lower concentrations may evoke the inhibitory effect. It is supposed that strenuous exercise induces NETs formation via shear and heat stress (Beiter et al., 2015). NETs formation involves activation of NADPH oxidase, mitochondrial respiratory chain, and increased ROS production (Quindry et al., 2003) that are postulated to be responsible for the increase in rLBCL just after exercise (Chmielecki et al., 2022). Therefore, the inhibitory effect of IL-4 on NETs formation seems to be the most probable explanation of the negative correlation between pre-exercise IL-4 and just-after-exercise ΔrLBCL.

4.2 Association of pre-exercise concentrations of other cytokines with rLBCL increment just after exercise

IL-8 and TNF-α can prime PMNs for enhanced ROS production in response to various agonists, including platelet activating factor (PAF), n-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP), complement component 5a (C5a) (You et al., 1991; Baggiolini and Clark-Lewis, 1992; Bajaj et al., 1992; Wozniak et al., 1993; Lloyds et al., 1995; Gougerot-Podicalo et al., 1996; Decleva et al., 2002; Guichard et al., 2005). Moreover, 2 h incubation of PMNs from healthy subjects with IL-8 and TNF-α at concentrations of 100 ng/mL and 8 ng/mL, respectively, induced NETs formation, which was accompanied by increased ROS production *in vitro* (Keshari et al., 2012). Therefore, we supposed that baseline circulating levels of IL-8 or TNF-α can affect the post-exercise increase in ROS production by PMNs. Surprisingly, there were no association between pre-exercise and just-after-exercise plasma concentrations of these cytokines and ΔrLBCL just after exercise. The following reasons may explain these negative results: (A)—concentrations of IL-8 and TNF-α used for induction of NETs formation were many times-higher than those occurred *in vivo* in healthy subjects (Kleiner et al., 2013); (B)—increase in plasma cell-free nuclear DNA reflecting NETs formation was observed within several minutes from the onset of strenuous exercise (Breitbach et al., 2014), and, therefore, initial signals switching on this process have rather physical (shear stress related to increased cardiac output and blood flow, and perhaps an increase in body temperature) but not inflammatory nature.

Jogging on a treadmill at 70% of maximum heart rate for 15 min resulted in an increase in the core temperature of the triceps surae to 39°C (Wirth et al., 1998). Preincubation of isolated human PMNs for 1 h at 39.5°C accelerated PMA- and *Pseudomonas* aeruginosa-induced NETs formation (Keitelman et al., 2019). In a working skeletal muscle, alternating blood vessels compression and dilation result in large changes of intramuscular blood flow velocity (Osada et al., 2015) and perhaps shear stress. More, elevated shear stress or turbulent blood flow can promote interaction of platelets with PMNs involving von Willebrand factor and binding of platelet αIIbβ3 to SLC44A2 on PMNs promoting mechanosensitive-dependent formation of NETs (Constantinescu-Bercu et al., 2020). On the other hand, in an *in vitro* microfluidic model of sterile thrombotic occlusion, increased rheological forces triggered NETs formation that was insensitive to inhibitors of platelet-PMNs adhesion (Yu et al., 2018). This may also explain the weak negative association between pre-exercise concentration of anti-inflammatory IL-10 and ΔrLBCL just after exercise.

4.3 Associations of plasma concentrations of monitored cytokines with rLBCL decrement at 1, 3, and 24 h post-exercise

The increase in rLBCL noted just after exercise was transient and from 1 h post-exercise till the end of observations at 24 h post-exercise, a significant decrease in rLBCL was noted in comparison to pre-exercise value. Since IL-8 and TNF-α can prime neutrophils for enhanced ROS production (You et al., 1991; Baggiolini and Clark-Lewis, 1992; Bajaj et al., 1992; Wozniak et al., 1993; Lloyds et al., 1995; Gougerot-Podicalo et al., 1996; Decleva et al., 2002; Guichard et al., 2005), we expected that subjects with higher plasma concentrations of these cytokines would have smaller post-exercise decrements in rLBCL (Δ-rLBCL). However, the Spearman's ρ analysis did not reveal such associations. These negative results can be explained by (A)—a transient nature of post-exercise increase in plasma IL-8 and TNF-α; (B)—*in vivo* concentrations of these cytokines are substantially lower then their concentration that evoked priming effect on PMNs *in vitro* (You et al., 1991; Baggiolini and Clark-Lewis, 1992; Bajaj et al., 1992; Wozniak et al., 1993; Lloyds et al., 1995; Gougerot-Podicalo et al., 1996; Decleva et al., 2002; Guichard et al., 2005); (C)—there are numerous other substances that can *in vivo* counterbalance these two pro-inflammatory cytokines: for instance, circulating catecholamines that rose during exhaustive exercise were reported to inhibit NETs formation (Vogelgesang et al., 2017); (D)—at 3 h post-exercise the blood phagocyte count increased by about 3-times (Chmielecki et al., 2022), indicating that a new population of these cells from marginated pool and bone marrow entered the circulation with probably different responsiveness to IL-8 and TNF-α in comparison to pre-exercise cells.

Exercise caused the highest increase in IL-10 that was noted just after and at 1 h post-exercise. This cytokine has anti-inflammatory activity and inhibits ROS production from human PMNs. Thus, one may expect that sportsmen presenting higher increase in IL-10 would have greater decline in rLBCL at 1 h and later after exercise. Similarly, no such associations were found. This is in agreement with our previous suggestion that post-exercise decrease in rLBCL may result from an increased number of younger PMNs from bone marrow that have lower ability to produce ROS (Chmielecki et al., 2022). It should be noted that Spearman analysis of IL-4 plasma levels with rLBCL decrements always revealed negative value of ρ, and for pre- and just-after-exercise IL-4 and ΔrLBCL at 1 h post-exercise, the negative correlations reached borderline significance, in means that sportsmen with higher pre-exercise concentrations of IL-4 would have a deeper decrement of rLBCL at 1 h post-exercise (Figure 9). In an animal model of antibody-induced arthritis, IL-4 suppressed the egress of PMNs from bone marrow and their migration to inflamed joints (Panda et al., 2020). Assuming that IL-4 can inhibit exercise-induced exit of young PMNs from bone marrow in humans, this

action could have a completely opposite effect: higher pre-exercise IL-4 would cause a lower post-exercise increment in the number of young immature circulating PMNs and a lower decrement of rLBCL at 1 h post-exercise. To check this hypothesis, we additionally analyzed associations between pre-exercise plasma concentrations of IL-4 and exercise-induced increment in the number of circulating phagocytes just after, and at 1, 3, and 5 h post-exercise. At any time point, no significant associations were found (Spearman's ρ ranged from −0.04 to −0.26, $p > 0.3$). Therefore, the plasma IL-4 effect on exercise-induced movement of blood phagocytes (namely, PMNs) from bone marrow into the bloodstream seems less likely. On the other hand, addition of IL-4 to suspensions of isolated human PMNs preactivated with INF-γ or TNF-α resulted in inhibition of hexose monophosphate shunt, which is involved in ROS production (Bober et al., 2000). It is possible that post-exercise PMNs, conditioned by various mediators released in response to exercise, could be inhibited by circulating IL-4, explaining the afore-mentioned negative correlation between IL-4 and rLBCL decrement at 1 h post-exercise.

5 Conclusion

To the best of our knowledge, this is the first study conducted in such strictly monitored conditions which includes kinetics and control (reference day). Furthermore, the observation and correlation of selected cytokins have not been performed before. This article may be of interest to a physiologist, sports coaches, but also for scientists who wonder about the relationship between exercise and physiological stress."

We found that exhaustive exercise caused transient increase in plasma concentration of IL-8, IL-10 and TNF-α in healthy amateur sportsmen. Although these cytokines can affect the activity of PMNs, no association of these cytokine plasma levels (pre- and post-exercise) with biphasic changes of rLBCL (increase just after exercise and decrease at subsequent time-points), reflecting ROS production by blood phagocytes, was noted. Plasma concentrations of IL-4 were not altered by the exhaustive treadmill run, however pre- and just-after-exercise concentrations of this cytokine were negatively associated with the increment in rLBCl just after exercise. Moreover, pre- and just-after- exercise plasma IL-4 levels tended to negatively correlate (borderline significance) with the decrease of rLBCL at 1 h post-exercise. This suggests that IL-4 can prevent excessive ROS production by blood phagocytes just after an exhaustive exercise and augment the second phase consisting in suppression of oxidants generation post-exercise. Therefore, circulating IL-4 can be involved in maintaining a proper balance between oxidants and antioxidants during strenuous exercise and post-exercise recovery. However, confirmation of this hypothesis requires further studies. In conclusion, despite the observed increment in some pro-inflammatory cytokines, the rise was transient and compensated by the increase in anti-inflammatory cytokines. Therefore, it seems that exercise, even to exhaustion, is relatively safe for people who practice sport.

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 humans were approved by the Medical University of Lodz Ethics Committee (RNN/118/17/KE). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization: DN and AC; methodology: DN and AC; conducted experiments: AC and KB; participant safety during exercise: SG and KK; performed data analysis, statistics: RS and AC; writing the original draft preparation: AC, DN, and RS; writing, review, and editing: DN, AC, RS, GP, and HJ; visualization, RS and AC; supervision: DN; funding acquisition: DN and RS. 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.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2023.1186296/full#supplementary-material

References

Baggiolini, M., and Clark-Lewis, I. (1992). Interleukin-8, a chemotactic and inflammatory cytokine. *FEBS Lett.* 307, 97–101. doi:10.1016/0014-5793(92)80909-z

Bajaj, M. S., Kew, R. R., Webster, R. O., and Hyers, T. M. (1992). Priming of human neutrophil functions by tumor necrosis factor: enhancement of superoxide anion

generation, degranulation, and chemotaxis to chemoattractants C5a and F-Met-Leu-Phe. *Inflammation* 16, 241–250. doi:10.1007/BF00918813

Beiter, T., Fragasso, A., Hartl, D., and Nieß, A. M. (2015). Neutrophil extracellular traps: a walk on the wild side of exercise immunology. *Sports Med.* 45, 625–640. doi:10.1007/s40279-014-0296-1

Bialasiewicz, P., Prymont-Przyminska, A., Zwolinska, A., Sarniak, A., Wlodarczyk, A., Krol, M., et al. (2014). Addition of strawberries to the usual diet decreases resting chemiluminescence of fasting blood in healthy subjects – possible health-promoting effect of these fruits consumption. *J. Am. Coll. Nutr.* 33, 274–287. doi:10.1080/07315724.2013.870502

Bialasiewicz, P., Prymont-Przyminska, A., Zwolinska, A., Sarniak, A., Wlodarczyk, A., Krol, M., et al. (2018). Sour cherries but not apples added to the regular diet decrease resting and fMLP-stimulated chemiluminescence of fasting whole blood in healthy subjects. *J. Am. Coll. Nutr.* 37, 24–33. doi:10.1080/07315724.2017.1354739

Bober, L. A., Rojas-Triana, A., Jackson, J. V., Leach, M. W., Manfra, D., Narula, S. K., et al. (2000). Regulatory effects of interleukin-4 and interleukin-10 on human neutrophil function *ex vivo* and on neutrophil influx in a rat model of arthritis. *Arthritis Rheum.* 43, 2660–2667. doi:10.1002/1529-0131(200012)43:12<2660::AID-ANR5>3.0.CO;2-4

Breitbach, S., Tug, S., and Simon, P. (2012). Circulating cell-free DNA: an up-coming molecular marker in exercise physiology. *Sports Med.* 42, 565–586. doi:10.2165/11631380-000000000-00000

Breitbach, S., Sterzing, B., Magallanes, C., Tug, S., and Simon, P. (2014). Direct measurement of cell-free DNA from serially collected capillary plasma during incremental exercise. *J. Appl. Physiol.* 117, 119–130. doi:10.1152/japplphysiol.00002.2014

Cabral-Santos, C., Gerosa-Neto, J., Inoue, D., Panissa, V. L. G., Gobbo, L. A., Zagatto, A. M., et al. (2015). Similar anti-inflammatory acute responses from moderate-intensity continuous and high-intensity intermittent exercise. *J. Sports Sci. Med.* 14, 849–856.

Cabral-Santos, C., Junior, E. A. D. L., Fernandes, I. M. D. C., Pinto, R. Z., Rosa-Neto, J. C., Bishop, N. C., et al. (2019). Interleukin-10 responses from acute exercise in healthy subjects: a systematic review. *J. Cell. Physiol.* 234, 9956–9965. doi:10.1002/jcp.27920

Capsoni, F., Minonzio, F., Ongari, A. M., Carbonelli, V., Galli, A., and Zanussi, C. (1997). Interleukin-10 down-regulates oxidative metabolism and antibody-dependent cellular cytotoxicity of human neutrophils. *Scand. J. Immunol.* 45, 269–275. doi:10.1046/j.1365-3083.1997.d01-393.x

Chaves, M. M., Silvestrini, A. A., Silva-Teixeira, D. N., and Nogueira-Machado, J. A. (1996). Effect *in vitro* of gamma interferon and interleukin10 on generation of oxidizing species by human granulocytes. *Inflamm. Res.* 45, 313–315. doi:10.1007/BF02252942

Cheng, A. J., Jude, B., and Lanner, J. T. (2020). Intramuscular mechanisms of overtraining. *Redox Biol.* 35, 101480. doi:10.1016/j.redox.2020.101480

Chmielecki, A., Bortnik, K., Galczynski, S., Padula, G., Jerczynska, H., Stawski, R., et al. (2022). Exhaustive exercise increases spontaneous but not fMLP-induced production of reactive oxygen species by circulating phagocytes in amateur sportsmen. *Biol. (Basel)* 11, 103. doi:10.3390/biology11010103

Constantinescu-Bercu, A., Grassi, L., Frontini, M., Salles-Crawley, I. I., Woollard, K., and Crawley, J. T. (2020). Activated $\alpha_{IIb}\beta_3$ on platelets mediates flow-dependent NETosis via SLC44A2. *Elife* 21, e53353. doi:10.7554/eLife.53353

Dang, P. M. C., Elbim, C., Marie, J. C., Chiandotto, M., Gougerot-Pocidalo, M. A., and El-Benna, J. (2006). Anti-inflammatory effect of interleukin-10 on human neutrophil respiratory burst involves inhibition of GM-CSF-induced p47PHOXphosphorylation through a decrease in ERK1/2 activity. *FASEB J.* 20, 1504–1506. doi:10.1096/fj.05-5395fje

Decleva, E., Dri, P., Menegazzi, R., Busetto, S., and Cramer, R. (2002). Evidence that TNF-induced respiratory burst of adherent PMN is mediated by integrin αLβ2. *J. Leukoc. Biol.* 72, 718–726. doi:10.1189/jlb.72.4.718

Egholm, C., Heeb, L. E. M., Impellizzieri, D., and Boyman, O. (2019). The regulatory effects of interleukin-4 receptor signaling on neutrophils in type 2 immune responses. *Front. Immunol.* 10, 2507. doi:10.3389/fimmu.2019.02507

Gougerot-Podicalo, M. A., Elbim, C., and Chollet-Martin, S. (1996). Modulation of the oxidative burst of human neutrophils by pro- and anti-inflammatory cytokines. *Pathol. Biol. Paris.* 44, 36–41.

Guichard, C., Pedruzzi, E., Dewas, C., Fay, M., Pouzet, C., Bens, M., et al. (2005). Interleukin-8-induced priming of neutrophil oxidative burst requires sequential recruitment of NADPH oxidase components into lipid rafts. *J. Biol. Chem.* 280, 37021–37032. doi:10.1074/jbc.M506594200

Heeb, L. E. M., Egholm, C., and Boyman, O. (2020). Evolution and function of interleukin-4 receptor signaling in adaptive immunity and neutrophils. *Genes Immun.* 21, 143–149. doi:10.1038/s41435-020-0095-7

Hoppenbrouwers, T., Autar, A. S. A., Sultan, A. R., Abraham, T. E., van Cappellen, W. A., Houtsmuller, A. B., et al. (2017). *In vitro* induction of NETosis: comprehensive live imaging comparison and systematic review. *PLOS One* 12, 0176472. doi:10.1371/journal.pone.0176472

Impellizzieri, D., Ridder, F., Raeber, M. E., Egholm, C., Woytschak, J., Kolios, A. G. A., et al. (2019). IL-4 receptor engagement in human neutrophils impairs their migration and extracellular trap formation. *J. Allergy. Clin. Immunol.* 144, 267–279. doi:10.1016/j.jaci.2019.01.042

Kakanis, M. W., Peake, J., Brenu, E. W., Simmonds, M., Gray, B., and Marshall-Gradisnik, S. M. (2014). T helper cell cytokine profiles after endurance exercise. *J. Interferon Cytokine Res.* 34, 699–706. doi:10.1089/jir.2013.0031

Keitelman, I. A., Sabbione, F., Shiromizu, C. M., Giai, C., Fuentes, F., Rosso, D., et al. (2019). Short-term fever-range hyperthermia accelerates NETosis and reduces pro-inflammatory cytokine secretion by human neutrophils. *Front. Immunol.* 10, 2374. doi:10.3389/fimmu.2019.02374

Keshari, R. S., Jyoti, A., Dubey, M., Kothari, N., Kohli, M., Bogra, J., et al. (2012). Cytokines induced neutrophil extracellular traps formation: implication for the inflammatory disease condition. *PLOS One* 7, e48111. doi:10.1371/journal.pone.0048111

Kleiner, G., Marcuzzi, A., Zanin, V., Monasta, L., and Zauli, G. (2013). Cytokine levels in the serum of healthy subjects. *Mediat. Inflamm.* 2013, 434010. doi:10.1155/2013/434010

Kukovetz, E. M., Bratschitsch, G., Hofer, H. P., Egger, G., and Schaur, R. J. (1997). Influence of age on the release of reactive oxygen species by phagocytes as measured by a whole blood chemiluminescence assay. *Free Radic. Biol. Med.* 22, 433–438. doi:10.1016/s0891-5849(96)00334-6

Lloyds, D., Brindle, N. P., and Hallett, M. B. (1995). Priming of human neutrophils by tumour necrosis factor-alpha and substance P is associated with tyrosine phosphorylation. *Immunology* 84, 220–226.

Neuberger, E. W. I., Sontag, S., Brahmer, A., Philippi, K. F. A., Radsak, M. P., Wagner, W., et al. (2022). Physical activity specifically evokes release of cell-free DNA from granulocytes thereby affecting liquid biopsy. *Clin. Epigenetics.* 14, 29. doi:10.1186/s13148-022-01245-3

Nieman, D. C., and Pence, B. D. (2020). Exercise immunology: future directions. *J. Sport. Health Sci.* 9, 432–445. doi:10.1016/j.jshs.2019.12.003

Osada, T., Mortensen, S. P., and Rådegran, G. (2015). Mechanical compression during repeated sustained isometric muscle contractions and hyperemic recovery in healthy young males. *J. Physiol. Anthropol.* 34, 36. doi:10.1186/s40101-015-0075-1

Padilha, C. S., Figueiredo, C., Minuzzi, L. G., Chimin, P., Deminice, R., Krüger, K., et al. (2021). Immunometabolic responses according to physical fitness status and lifelong exercise during aging: new roads for exercise immunology. *Ageing Res. Rev.* 68, 101341. PMID: 33839332. doi:10.1016/j.arr.2021.101341

Panda, S. K., Wigerblad, G., Jiang, L., Jiménez-Andrade, Y., Iyer, V. S., Shen, Y., et al. (2020). IL-4 controls activated neutrophil FcγR2b expression and migration into inflamed joints. *Proc. Natl. Acad. Sci. U. S. A.* 117, 3103–3113. doi:10.1073/pnas.1914186117

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.

Quindry, J. C., Stone, W. L., King, J., and Broeder, C. E. (2003). The effects of acute exercise on neutrophils and plasma oxidative stress. *Med. Sci. Sports Exerc.* 35, 1139–1145. doi:10.1249/01.MSS.0000074568.82597.0B

Stawski, R., Walczak, K., Kosielski, P., Meissner, P., Budlewski, T., Padula, G., et al. (2017). Repeated bouts of exhaustive exercise increase circulating cell free nuclear and mitochondrial DNA without development of tolerance in healthy men. *PLOS One* 12, e0178216. doi:10.1371/journal.pone.0178216

Stawski, R., Walczak, K., Perdas, E., Wlodarczyk, A., Sarniak, A., Kosielski, P., et al. (2019). Decreased integrity of exercise-induced plasma cell free nuclear DNA—negative association with the increased oxidants production by circulating phagocytes. *Sci. Rep.* 9, 15970. doi:10.1038/s41598-019-52409-w

Stawski, R., Walczak, K., Perdas, E., Prymont-Przyminska, A., Zwolinska, A., Kosielski, P., et al. (2021). Increased circulating H3 histone in response to repeated bouts of exercise does not associate with parallel alterations of cell-free DNA. *Biol. (Basel)* 10, 181. doi:10.3390/biology10030181

Suzuki, K. (2019). Characterization of exercise-induced cytokine release, the impacts on the body, the mechanisms and modulations. *Int. J. Sports Exerc. Med.* 5, 122. doi:10.23937/2469-5718/1510122

Tanaka, H., Monahan, K. D., and Seals, D. R. (2001). Age-predicted maximal heart rate revisited. *J. Am. Coll. Cardiol.* 37 (1), 153–156. doi:10.1016/s0735-1097(00)01054-8

Tanskanen, M., Atalay, M., and Uusitalo, A. (2010). Altered oxidative stress in overtrained athletes. *J. Sports Sci.* 28, 309–317. doi:10.1080/02640410903473844

Velders, M., Treff, G., Machus, K., Bosnyák, E., Steinacker, J. M., and Schumann, U. (2014). Exercise is a potent stimulus for enhancing circulating DNase activity. *Clin. Biochem.* 47, 471–474. doi:10.1016/j.clinbiochem.2013.12.017

Vogelgesang, A., Lange, C., Blümke, L., Laage, G., Rümpel, S., Langner, S., et al. (2017). Ischaemic stroke and the recanalization drug tissue plasminogen activator interfere with

antibacterial phagocyte function. *J. Neuroinflammation.* 14, 140. doi:10.1186/s12974-017-0914-6

Vorobjeva, N. V., and Chernyak, B. V. (2020). NETosis: molecular mechanisms, role in physiology and pathology. *Biochemistry* 85, 1178–1190. doi:10.1134/S0006297920100065

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

Wirth, V. J., Van Lunen, B. L., Mistry, D., Saliba, E., and McCue, F. C. (1998). Temperature changes in deep muscles of humans during upper and lower extremity exercise. *J. Athl. Train.* 33, 211–215.

Wozniak, A., Betts, W. H., Murphy, G. A., and Rokicinski, M. (1993). Interleukin-8 primes human neutrophils for enhanced superoxide anion production. *Immunology* 79, 608–615.

Yamada, M., Suzuki, K., Kudo, S., Totsuka, M., Simoyama, T., Nakaji, S., et al. (2000). Effect of exhaustive exercise on human neutrophils in athletes. *Luminescence* 15, 15–20. doi:10.1002/(SICI)1522-7243(200001/02)15:1<15::AID-BIO570>3.0.CO;2-O

You, A., Kitagawa, S., Kasahara, T., Matsushima, K., Saito, M., and Takaku, F. (1991). Stimulation and priming of human neutrophils by interleukin-8: cooperation with tumor necrosis factor and colony-stimulating factors. *Blood* 78, 2708–2714. doi:10.1182/blood.v78.10.2708.bloodjournal78102708

Yu, X., Tan, J., and Diamond, S. L. (2018). Hemodynamic force triggers rapid NETosis within sterile thrombotic occlusions. *J. Thromb. Haemost.* 16, 316–329. doi:10.1111/jth.13907

frontiers | Frontiers in Physiology

TYPE Review
PUBLISHED 15 January 2024
DOI 10.3389/fphys.2023.1332104

OPEN ACCESS

EDITED BY
Simone Luti,
University of Florence, Italy

REVIEWED BY
Liliana C. Baptista,
University of Alabama at Birmingham, United States
Samuel Honório,
Polytechnic Institute of Castelo Branco, Portugal
Bernardo A. Petriz,
University Centre of the Federal District, Brazil

*CORRESPONDENCE
Jinglun Yu,
lun199517@126.com
Lijun Yin,
Jackyin93@126.com

RECEIVED 02 November 2023
ACCEPTED 27 December 2023
PUBLISHED 15 January 2024

CITATION
Qi S, Li X, Yu J and Yin L (2024), Research advances in the application of metabolomics in exercise science.
Front. Physiol. 14:1332104.
doi: 10.3389/fphys.2023.1332104

Research advances in the application of metabolomics in exercise science

Shuo Qi[1], Xun Li[1], Jinglun Yu[2]* and Lijun Yin[3]*

[1]School of Sport and Health, Shandong Sport University, Jinan, China, [2]School of Exercise and Health, Shanghai University of Sport, Shanghai, China, [3]School of Sport, Shenzhen University, Shenzhen, China

Exercise training can lead to changes in the metabolic composition of an athlete's blood, the magnitude of which depends largely on the intensity and duration of exercise. A variety of behavioral, biochemical, hormonal, and immunological biomarkers are commonly used to assess an athlete's physical condition during exercise training. However, traditional invasive muscle biopsy testing methods are unable to comprehensively detect physiological differences and metabolic changes in the body. Metabolomics technology is a high-throughput, highly sensitive technique that provides a comprehensive assessment of changes in small molecule metabolites (molecular weight <1,500 Da) in the body. By measuring the overall metabolic characteristics of biological samples, we can study the changes of endogenous metabolites in an organism or cell at a certain moment in time, and investigate the interconnection and dynamic patterns between metabolites and physiological changes, thus further understanding the interactions between genes and the environment, and providing possibilities for biomarker discovery, precise training and nutritional programming of athletes. This paper summaries the progress of research on the application of exercise metabolomics in sports science, and looks forward to the future development of exercise metabolomics, with a view to providing new approaches and perspectives for improving human performance, promoting exercise against chronic diseases, and advancing sports science research.

KEYWORDS

metabolomics, exercise, metabolism, nutrition, biomarkers

Introduction

A biomarker is a biological characteristic that can be measured and evaluated in an organism or a biological sample, and can be used to indicate the physiological state of an organism, disease risk, disease progression or treatment effect, and other information (Qiu et al., 2023). Biomarkers can be molecules, cells, tissues, or physiological indicators, etc. Common biomarkers include genes, proteins, metabolites, hormones, and cell surface markers (Di Minno et al., 2022). Metabolomics is the quantitative analysis of small molecular weight metabolites (molecular weight <1,500 Da) in organisms, such as carbohydrates, amino acids, organic acids, nucleotides, and lipids, using mass spectrometry or magnetic resonance spectroscopy, and by studying the pattern of change of organisms or endogenous metabolites at a given moment (Kelly et al., 2020; Schranner et al., 2020; Belhaj et al., 2021; Shimada et al., 2021; Khoramipour et al., 2022). It is possible to investigate the interconnection and dynamic pattern of metabolites and physiological and pathological changes (Kelly et al., 2020; Kistner et al., 2021). Metabolomics reflects the genome, transcriptome, and proteome, as well as their

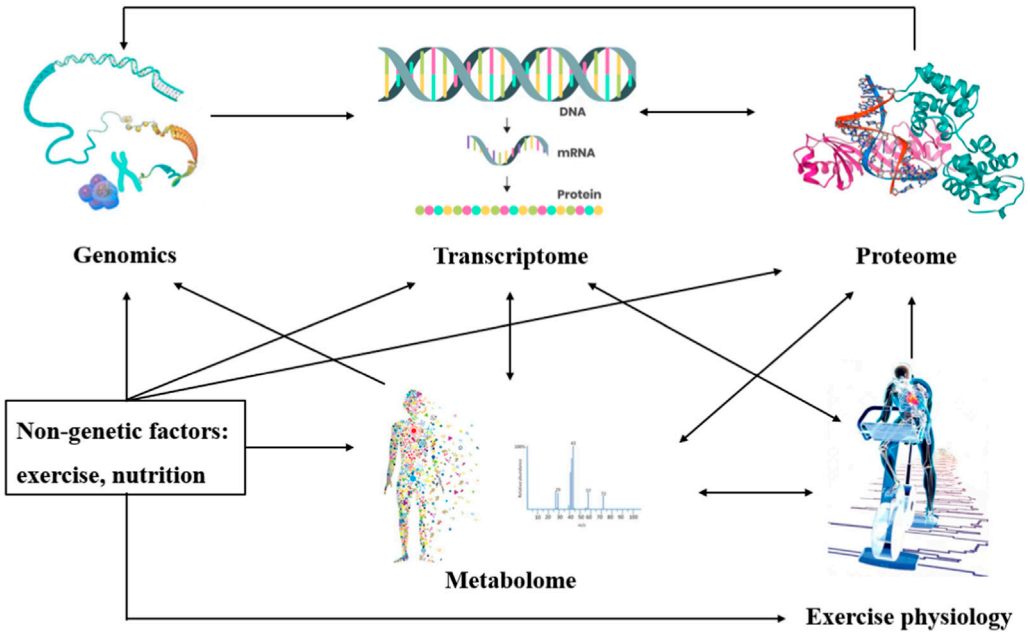


FIGURE 1
Interconnections between metabolomics and the environment. Metabolomics reflects the genome, transcriptome, and proteome and their interactions with the environment.

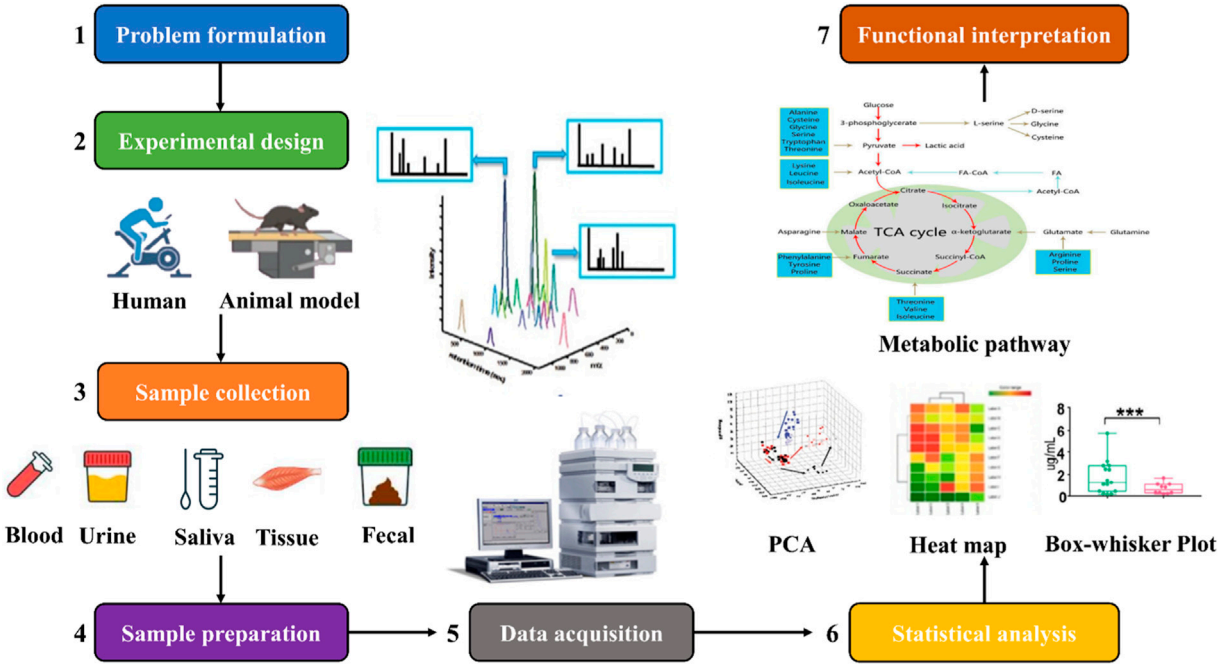


FIGURE 2
Workflow of exercise metabolomics studies. The workflow of exercise metabolomics research mainly includes problem formulation, study design, sample collection, sample preparation, data collection, statistical analysis, and functional interpretation.

interactions with the environment, and provides an ideal way to measure organismal phenotypes (Figure 1). Metabolomics data can provide useful insights into the biological effects of exercise, drug therapy, nutritional interventions, and more. Over the past decades, metabolomics has become a powerful tool for studying metabolic processes, identifying potential biomarkers, and deciphering metabolic reprogramming in various diseases to reveal the underlying mechanisms of relevant metabolic diseases (Belhaj et al., 2021).

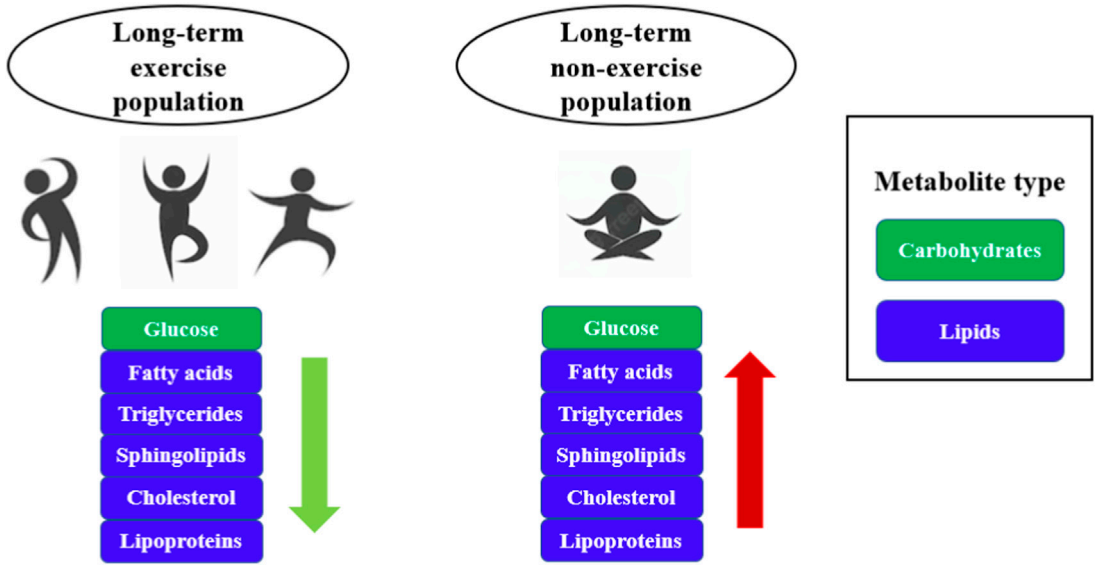


FIGURE 3
Comparison of metabolites between the long-term exercising population and the non-exercising population. The workflow of exercise metabolomics research mainly includes problem formulation, study design, sample collection, sample preparation, data collection, statistical analysis, and functional interpretation.

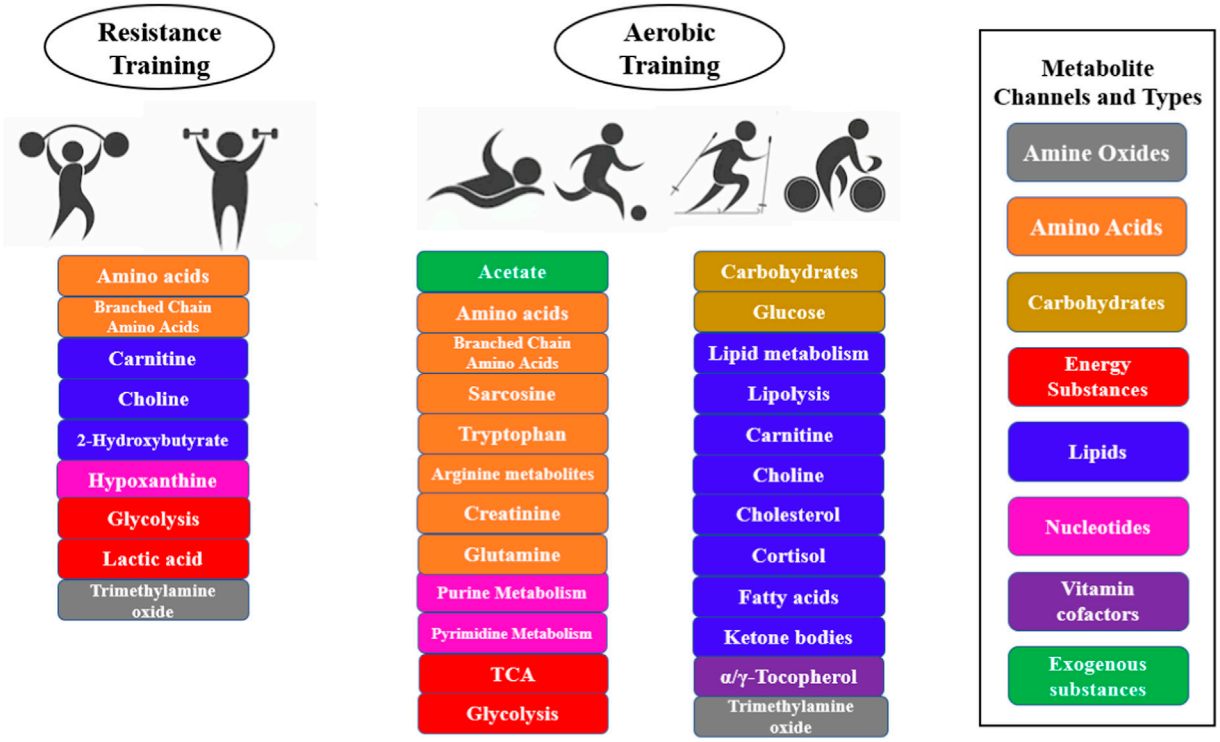


FIGURE 4
Metabolites, metabolic pathways of different exercise intensity category interventions.

Exercise can cause changes in the metabolism of many organs and tissues of the body, both acute and prolonged exercise, causing changes and adaptations in the body's material metabolism and energy metabolism. Meanwhile, metabolites also regulate cellular signal release, energy transfer, and intercellular communication in the organism (Monnerat et al., 2020; Schranner et al., 2020; Belhaj et al., 2021; Klein et al., 2021; Martins Conde et al., 2021). However, exercise physiology has traditionally only been able to study a small

TABLE 1 Advantages and disadvantages of different chemical analysis methods.

Analytical platform	Advantages	Disadvantages
Nuclear magnetic resonance (NMR)	Fast Analysis	Low sensitivity
	High resolution technology	Small library of reference compounds
	No derivatisation required	More than one peak per metabolite
	Simple preparation	Limited to hydrophilic molecules
	Highly reproducible	Expensive instrumentation
	Low cost	
	Structure can be determined	
	Fully automatable	
	Non-destructive methods	
Gas chromatography–mass spectrometry (GC–MS)	High sensitivity	Lower yield
	High resolution	Requires chemical derivatisation
	Large linear absorption range	Not suitable for thermally unstable compounds
	Suitable for volatile compounds	High molecular weight
	Large library of commercial and public reference spectra	Only for compounds that can volatilise
	Highly reproducible	Complicated preparation process
	Mostly automatable	
Liquid chromatography–mass spectrometry (LC–MS)	Usually no derivatisation required	Low throughput
	Can be used with a variety of separation methods	Limited library of reference spectra
	Multiple samples can be analyzed simultaneously	Expensive instrumentation
	Suitable for a wide range of compounds (polar and non-polar)	Chromatographic separation required
	Most sensitive metabolomics technology	Advanced training required
	Good automation capabilities	Destructive methods

TABLE 2 Effects of different exercise durations on key metabolites.

Duration of different exercises	Changes in key metabolites
Acute exercise of short duration	Leucine, isoleucine, asparagine, methionine, lysine, glutamic acid, glutamine, alanine, dimethylguanidinovaleric acid and other metabolites ↓
Long period of exercise	Glucose, isoleucine, fatty acids, triglycerides, cholesterol, sphingolipids, lipoproteins and other metabolites ↓

number of genes, proteins, and metabolites and their responses or adaptations to exercise, with no more than 12 metabolites measured using traditional methods and only one to two metabolic pathways at a time, failing to comprehensively detect exercise-induced physiological changes in tissues or metabolic pathways (Belhaj et al., 2021; Khoramipour et al., 2022). The number of metabolites in the human body exceeds 110,000 compounds and the number of metabolic signaling pathways in the human body exceeds 40,000 (Wishart et al., 2020). In addition, invasive muscle biopsies are required to collect metabolic data in exercise physiology studies (Belhaj et al., 2021; Tokarz et al., 2021). While invasive muscle biopsies have successfully identified certain key metabolic pathways in the body, such as glycolysis and β-oxidation of free fatty acids, this invasive testing methodology limits the motivation of subjects to participate in the test and further limits the measurement of certain meaningful metabolic analyses, whereas the emergence of metabolomics has made it possible to conduct comprehensive, high-throughput, minimally or non-invasive metabolic studies in the field of exercise physiology (Castro et al., 2020). This review briefly introduces the exercise metabolomics technology and workflow, focuses on the research progress of exercise metabolomics applied in the field of sports science, and looks forward to the future development direction of exercise metabolomics. The technique provides researchers with an effective research tool, which helps to improve the practical ability and depth of theoretical understanding of sports performance and chronic disease exercise control.

Introduction to exercise metabolomics

With the continuous development of histological techniques, exercise physiology is increasingly using metabolomics to probe organismal phenotypes, reveal metabolic pathways through the measurement of endogenous compounds, and identify biomarkers associated with exercise performance and fatigue, which has been termed "exercise metabolomics" (Kelly et al., 2020; Zhou et al., 2021). In 2007, Pohjanen et al. (2007) introduced exercise metabolomics to exercise science by performing 90 min of stationary cycling on 24 healthy men, collecting blood samples for gas chromatography-mass spectrometry (GC-MS) analysis, and identifying 420 metabolites, of which 34 were significantly altered, with an emphasis on the role of the most valuable biomarkers (glycerol and asparagine), which demonstrated, for the first time, the potential of non-targeted GC-MS metabolomics to provide a useful tool for the identification of metabolic pathways associated with exercise performance and fatigue. Metabolomics by mass spectrometry may provide a comprehensive and unbiased approach to studying the metabolic effects of exercise interventions (Pohjanen et al., 2007). Currently, the most commonly used biological samples in exercise metabolomics studies are blood and urine, and most studies use non-targeted metabolomics techniques, with mass spectrometry being the most commonly used detection and analysis platform in exercise metabolomics studies.

The main question in exercise science research is to understand how exercise induces physiological adaptations in the body, such as an increase in muscle strength or aerobic metabolic capacity, and how these adaptations affect health. But what are the molecular network mechanisms and metabolic pathways that govern how humans adapt to exercise and gain health benefits (Sakaguchi et al., 2019; Blackburn et al., 2020; Febvey-Combes et al., 2021; Babu et al., 2022)? These questions remain to be fully elucidated, and the study of exercise metabolomics will greatly enrich the understanding of these molecular network mechanisms and metabolic pathways.

Workflow of exercise metabolomics studies

As shown in Figure 2, the workflow of exercise metabolomics research includes 1) identifying the biological question of the study; 2) developing a study design based on the biological question of the study; 3) collecting experimental samples; 4) preparing the samples; 5) analyzing the samples and acquiring the data using one or more analytical platforms; 6) statistically analyzing compounds based on the biological question and experimental design to determine metabolic differences between different groups of samples; 7) researchers use software tools and databases to integrate the detected compounds with the biological context to further enable metabolic pathway enrichment analysis, metabolite mapping, and visualization, which can help inform future research questions and experimental designs (Belhaj et al., 2021; Khoramipour et al., 2022).

Sample collection and sample preparation

In human metabolomics research, the most common sample types are blood, urine, saliva, sweat, and fecal samples, with muscle biopsies and other types of tissue biopsies accounting for a lower percentage, and endogenous metabolites in the samples better reflect the physiological changes in the organism (Kelly et al., 2020; Khoramipour et al., 2022). Sample type, sample quantity, and sample storage conditions are the keys to metabolomics experiments, each biological sample has advantages and disadvantages, the most commonly used types of biological samples in exercise metabolomics research are blood and urine, and the collection method is minimally invasive or non-invasive, and easy to be accepted by the subjects. Once collected, biological samples must be further processed or extracted to convert them into a state suitable for chemical analysis (Khoramipour et al., 2022).

Chemical analysis platform and data analysis

Chemical analysis platforms used for sample characterization in metabolomics research include Gas Chromatography-Mass Spectrometry (GC-MS), Nuclear Magnetic Resonance (NMR), and Liquid Chromatography-Mass Spectrometry (LC-MS) (Table 1) (Kelly et al., 2020; Nicolaides et al., 2021; Khoramipour et al., 2022). The process of data processing and information analysis in metabolomics mainly includes the analysis of data, extraction of biological information, and functional interpretation of biological connotations (Khoramipour et al., 2022). The data generated in metabolomics studies have multivariate characteristics, when the number of metabolites in a given sample reaches hundreds or thousands, multivariate analysis methods capable of dealing with related variables are required to achieve reliable comparisons between multiple samples based on the whole set of variables. For example, Principal Components Analysis (PCA), Partial Least Squares-Discriminant Analysis (PLS-DA), and Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) (Castro et al., 2020; Khoramipour et al., 2022).

Advances in exercise metabolomics research

There is a multifactorial dosage relationship between the effects of exercise on metabolic pathways, including the intensity of exercise, the duration of exercise, and the frequency of exercise (Hargreaves and Spriet, 2020; Osawa et al., 2021). These factors can strongly influence the metabolic changes in the organism after exercise. In turn, the type and program of exercise, the level of exercise, and even exercise nutrition can also affect the body's metabolism. In addition, the effect of exercise on the metabolomics of chronic diseases is also a current research hotspot, which provides new perspectives on the prevention and treatment of chronic diseases.

Effects of different exercise durations on body metabolism

There are some differences in the categories of metabolites induced in the body by different modes of exercise. For example, a short period of acute exercise can immediately cause changes in the metabolic pathways of skeletal muscle substrate utilization, and the changes in tricarboxylic acid (TCA) cycle metabolites are obvious after 1 h of exercise (Kelly et al., 2020; Tabone et al., 2021). Amino acids such as leucine, isoleucine, asparagine, methionine, lysine, glutamine, and alanine decreased significantly after 14 h of exercise, reflecting the large magnitude of changes in amino acid levels after acute exercise (Sakaguchi et al., 2019). Changes in plasma fatty acids, ketone bodies, bile acids, and triglycerides also showed changes that can last for several hours after acute exercise, eventually returning to pre-exercise levels (Sakaguchi et al., 2019). For example, weight lifting and dumbbell training, resistance exercises such as pull-ups. Sakaguchi et al. found that within 24 h of a short period of acute exercise in the body, Significant changes in metabolites such as carbohydrates, TCA circulating metabolites, fatty acids, carnitine, ketone bodies, amino acids, and their derivatives were found (Sakaguchi et al., 2019). Nayor et al. (2022) found that dimethylguanidinopentanoic acid and glutamate levels were reduced after a short period of acute exercise (Nayor et al., 2022). Therefore, short-duration acute exercise can cause more substantial changes in metabolites related to energy metabolism.

Kujala et al. (2013) compared amino acid levels in several groups of twins who had been exercising consistently for several decades and found that the fatty acid composition of the long-term exercising population gradually shifted from a saturated to an unsaturated state and that glucose and isoleucine levels were lower (Kujala et al., 2013). As shown in Figure 3, changes in metabolites such as glucose, fatty acids, and triglycerides were observed in both long-term exercising and long-term non-exercising populations, but higher levels of fatty acids, triglycerides and cholesterol existed in long-term non-exercising populations, which are prone to chronic metabolic diseases such as dyslipidemia, hypertension, cardiovascular disease, stroke, type 2 diabetes and metabolic syndrome (Mendham et al., 2021; Remie et al., 2021; McClain et al., 2022). However, people who exercise for a long period can accelerate the utilization of energy substances, reduce the accumulation of fat, and lower the levels of fatty acids, triglycerides, and cholesterol, which is conducive to the maintenance of healthy body weight as well as lowering the risk of chronic diseases (Table 2) (Tzimou et al., 2020; Bihlmeyer et al., 2021; Koay et al., 2021; Lemonakis et al., 2022). In the future, we will focus on the far-reaching effects of long-term exercise on weight loss and health.

Effects of different exercise intensities on body metabolism

The most common metabolic pathways induced by exercise in the body are changes in fatty acid metabolism, fat mobilization, lipolysis, TCA cycle, glycolysis, amino acid metabolism, carnitine metabolism, purine metabolism, and cholesterol metabolism (Kelly et al., 2020). Different exercise intensities have different effects on the body's metabolism, with low-intensity aerobic training being dominated by aerobic metabolic pathways, with increases in the TCA cycle, fatty acid metabolism and amino acid metabolic pathways, and high-intensity resistance training being dominated by anaerobic metabolic pathways, with an increase in glycolysis and purine metabolism pathways (Figure 4). The effect of exercise intensity on metabolic profiles was also present in outstanding athletes, Al-Khelaifi et al. (2018) collected blood samples from outstanding athletes in different sports and analyzed the changes in 743 metabolites based on an LC-MS platform, and found that outstanding athletes with low-intensity endurance training had higher levels of serum sex hormones (testosterone and progesterone), and lower levels of diacylglycerol and eicosanoids; while high-intensity strength-trained elite athletes had higher levels of phospholipids and xanthines (Al-Khelaifi et al., 2018). Aerobic training mainly includes, running, cycling, football, and endurance sports such as swimming. Resistance training usually consists of high load, low repetition muscle contractions during a race (Granacher et al., 2016). Examples include weightlifting training, polymetric training, or machine-based training that includes upper and lower body exercises such as squats, jumps, weighted sprints, push-ups, and pull-ups (Fiorenza et al., 2019). This type of training is known to promote metabolic changes that facilitate anaerobic processes and increase muscle strength. Exercises such as gymnastics, martial arts and rock climbing also exhibit a high resistance component. In addition, endurance and resistance components are often combined, for example, in exercise interventions that combine running with weight training. Many sports also have significant endurance and resistance components, such as sprinting, boxing and rugby.

The type of sport also affects the body's metabolic differences, such as marathons, track, boxing, cycling, football, rowing, rugby swimming, etc. There are differences in metabolic changes in different sports, and the reason for metabolic differences in different sports is mainly due to the different proportion of the energy supply system, such as weightlifters with the phosphate energy supply system, rowers with the glycolysis energy supply system, and marathon athletes with the aerobic oxidative energy supply system (Al-Khelaifi et al., 2018). Even the metabolism of athletes in different positions in the same sport varies, e.g., there are metabolic differences between football goalkeepers and field players participating in the same game, and these differences are likely to be caused by exercise intensity and exercise duration (Blackburn et al., 2020; Schader et al., 2020; Bester et al., 2021; Pugh et al., 2021). In addition, athletes in endurance sports have significantly increased levels of glycolytic products, TCA cycle intermediates, nucleotide metabolites, acylcarnitines, and branched-chain amino acids, which are frequently associated with aerobic metabolic pathways. Resistance training studies have shown significant increases in levels of creatine, choline, guanidine acetate, and hypoxanthine and decrease in creatinine levels in athletes in strength and explosive events, metabolites that are commonly associated with muscle growth, intracellular buffering, and methyl regulation (Khoramipour et al., 2022).

Effects of different levels of exercise on the body's metabolism

The level of exercise also affects the body's metabolic differences, and Enea et al. used metabolomics for the first time to differentiate

between metabolite changes in trained and untrained women, who underwent a 75% maximal oxygen uptake test, and then collected urine samples to analyze the metabolite changes based on an NMR platform, and found that the metabolites of creatine, lactic acid, pyruvic acid, alanine, β-hydroxybutyric acid, acetate, and hypoxanthine significant differences between groups (Enea et al., 2010).

Not only is there a difference in metabolism between trained and untrained individuals, but also the same athletes and different levels of exercise affect metabolism. Schader et al. (2020) found that slower marathon runners with lower levels of aerobic metabolism capacity had drastically altered levels of metabolites, with significant changes in phospholipids and amino acids (Schader et al., 2020). In contrast, the metabolomic alterations in good athletes were characterized by higher levels of phosphatidylcholine after the race (Høeg et al., 2020). San-Millan et al. (2020) found increased levels of circulating metabolites in TCA and elevated amplitude of lactate accumulation in good cyclists (San-Millán et al., 2020). In addition, Prado et al. collected urine samples from football players and analyzed their metabolic changes during competition based on an LC-MS platform, identifying 1,091 metabolites, of which 526 metabolites showed significant changes, including significant increases in the levels of glucose, uric acid urea, fatty acyls, carboxylic acids, steroids and steroid derivatives, and significant decreases in the levels of potassium (Khoramipour et al., 2022). Hudson et al. (2021) based on an NMR platform analyzed the metabolite changes in blood, urine, and saliva samples of outstanding rugby players, and found that the energy metabolism pathways of rugby, as a sport with high exercise intensity, mainly include glycolysis, TCA cycle, and gluconeogenesis (Hudson et al., 2021). Moreira et al. (2018) analyzed the urinary metabolites of outstanding swimmers and found that creatine, ketone bodies, phosphate, and nitrogen-containing compounds can be used as urinary metabolites to assess the outstanding swimmers' exercise performance, which can be accurately assessed. Athletes' performance, which can accurately assess their physiological status and provide a scientific basis for the development of athletes' training load programs (Moreira et al., 2018).

The effect of sports nutrition on the body's metabolism

Kirwan et al. (2009) found that post-exercise intake of sugars and caffeine and analysis of metabolite changes in blood samples based on an NMR platform revealed a significant decrease in blood glucose levels, a significant increase in ketone body levels, and a significant increase in plasma levels of lactic acid and alanine (required for gluconeogenesis), which is the first study of sports nutrition metabolomics (Kirwan et al., 2009). Kozlowska et al. (2020) explored the effects of beetroot juice supplementation on the metabolism of fencing, and urine samples were collected to identify changes in metabolites based on an LC-MS platform, and significant changes in the metabolism of tyrosine, tryptophan, epinephrine, and norepinephrine were detected, which can help provide a scientific basis for the development of training load programs for athlete (Kozlowska et al., 2020). Yan et al. (2018) investigated the modulatory effects of ginseng supplementation on the metabolic patterns of professional athletes and explored the mechanism of ginseng's antifatigue effects. Their metabolite analysis of blood samples from athletes based on a GC-MS platform revealed that American ginseng significantly modulated serum metabolism, significantly decreasing serum creatine kinase and blood urea nitrogen levels (Yan et al., 2018). Cronin et al. (2018) explored the effects of physical activity and protein intake on gut microbial composition and function, and genomics and metabolomics evaluations revealed significant changes in gut microbial composition and function with increased physical activity, with the gut virome significantly changing with increased physical activity in participants who received daily supplementation with whey protein (Cronin et al., 2018). Among participants receiving daily whey protein supplementation, the diversity of the gut virome changed significantly, suggesting that exercise and nutrition can significantly influence the composition and function of the gut microbiota. Therefore, metabolomics serves as an assessment tool to facilitate the design of personalized and fine-tuned exercise training and nutritional guidance programs for athletes, which can help to maximize athletic performance. In addition, Zhang et al. (2020) explored the effects of a 6-month exercise and dietary intervention on serum metabolites in men with insomnia symptoms, collecting blood samples from subjects for metabolite analysis based on a GC-MS platform, and found that the effects of exercise on sleep were mainly related to amino acid, carbohydrate and lipid metabolism, whereas the effects of diet on sleep were related to carbohydrate, lipid and organic acid metabolism (Zhang et al., 2020). Thus, metabolomics provides new insights into the effects of physical activity and diet on sleep quality.

Exercise metabolomics in chronic disease prevention and treatment research

Contrepois et al. (2020) analyzed more than 600 metabolite changes in blood samples collected based on an LC-MS platform using a variety of histological approaches (targeted and untargeted metabolomics, lipidomics, proteomics, and transcriptomics), and showed that exercise has a significant effect on energy metabolism, oxidative stress, inflammation, tissue repair and its regulatory pathways in diabetic patients (Contrepois et al., 2020). Shi et al. (2019) found that Exercise can alter myocardial and skeletal muscle metabolism in heart failure model rats, and the metabolic pathways of taurine and hypotaurine metabolism and carnitine synthesis have a certain regulatory effect on alleviating heart failure, thus providing an effective target for the treatment of patients with heart failure (Shi et al., 2019). Siopi et al. (2019) studied the effect of exercise training with different intensities on male patients with metabolic syndrome and collected blood samples to analyze the changes of metabolites based on the LC-MS platform (Siopi et al., 2019). They found that resistance training induced the strongest metabolic changes, and the metabolites of branched-chain amino acids, alanine, carnitine, choline, and betaine had larger changes, indicating that exercise has beneficial effects on important serum biomarkers in patients with metabolic syndrome, which can help optimize the exercise

guidelines for the people with risk of metabolic syndrome and improve the exercise prescription (Siopi et al., 2019). Palmnas et al. (2018) analyzed the blood samples of obese people based on the NMR platform and found that the obese people had the best metabolite changes. Blood samples and found that serine and glycine concentrations were lower in the obese population, which can help to find molecular targets for the treatment of chronic metabolic diseases in obese populations (Palmnäs et al., 2018). Liu et al. (2021) collected blood samples from children with metabolic syndrome and analyzed the changes in metabolites based on the LC-MS platform, and found that exercise combined with dietary interventions induced 59 metabolites (glycine, serine, and threonine metabolisms, nitrogen metabolism, TCA cycling, and phenylalanine, tyrosine, and tryptophan biosynthesis, etc.) to changes, thus providing early diagnostic biomarkers for the treatment of metabolic diseases such as obesity (Liu et al., 2021).

Analysis of metabolic pathways by exercise

Exercise training induces changes in the body's metabolic pathways such as lipid metabolism, TCA cycle, glycolysis, amino acid metabolism, carnitine metabolism, and purine metabolism (Kelly et al., 2020). Positive effects on cardiovascular health and mitochondrial biogenesis exist in populations that engage in chronic low-intensity aerobic training, where energy is produced through oxidative phosphorylation (Rivera-Brown and Frontera, 2012). Therefore, the activation of aerobic metabolic pathways and the increase in the TCA cycle, fatty acid β-oxidation metabolism and amino acid metabolism pathways, which allows for the presence of lower levels of fatty acids, triglycerides, and cholesterol in the organism, can accelerate the utilisation of energy substances and reduce the accumulation of fat, which is conducive to the maintenance of a healthy body weight as well as reducing the risk of chronic diseases, in addition to increasing the variety of energy substances burned during exercise (Pellegrino et al., 2022). Chronic metabolic adaptations in prolonged exercise populations typically affect metabolic pathways such as glycolysis, protein synthesis, amino acid consumption, and nucleotides.

In addition to improving muscle strength and metabolic health with short bursts of high-intensity resistance training, it also induces muscle hypertrophy. Resistance training is associated with metabolic changes that contribute to improved anaerobic capacity, muscle health, and glycolytic metabolism (Krzysztofik et al., 2019). Resistance training is mainly dominated by anaerobic metabolic pathways, with an increase in glycolytic and purine metabolic pathways. One of the most significant metabolic adaptations induced by resistance training is the increase in protein synthesis and depletion of amino acids, which are necessary to increase muscle mass (Shen et al., 2021; Gehlert et al., 2022). In addition, the pathways involved in nucleotide synthesis—the production of RNA, DNA and phospholipids required for cellular membranes—are activated (Pellegrino et al., 2022). There is an increase in the rate of ATP hydrolysis and the rate of nucleotide turnover, an increase in the accumulation of lactic acid in metabolites following acute resistance training and an increase in the ability to promote glycolytic metabolic adaptations (Gehlert et al., 2022). Metabolic adaptations in elite athletes are characterised by increased fuel substrate utilisation, fatty acid β-oxidation, oxidative stress, steroid biosynthesis and protein anabolic pathways (Cai et al., 2022).

Physical activity has many benefits for both physical and mental health, as studied through metabolomic analysis of metabolites released from tissues such as skeletal muscle, bone and liver. These metabolites can influence the body's metabolic adaptations and improve cardiovascular health, reduce inflammation and increase muscle mass. Aerobic training increases mitochondrial content and oxidative enzymes, while resistance training increases muscle fibres and glycolytic enzymes. Acute exercise leads to changes in amino acid metabolism, lipid metabolism and cellular energy metabolism as well as cofactor metabolism and vitamin metabolism. Chronic exercise leads to changes in amino acid metabolism, lipid metabolism and nucleotide metabolism and improves lipid metabolism, thereby improving cardiovascular risk factors and skeletal muscle adaptations. The study of exercise-induced metabolites is a growing field with the potential to reveal more metabolic mechanisms and tailor exercise programs for optimal health and exercise performance.

Potential limitations of exercise metabolomics

Many early exercise metabolomics studies lacked statistical rigor, e.g., extensive use of multivariate statistics, small sample sizes, and a single platform for metabolic analyses (Khoramipour et al., 2022). Furthermore, there were deficiencies in the sensitivity and specificity of the biomarkers used in most exercise metabolomics studies (Schranner et al., 2021). With the continuous advancement of histological technologies, metabolomics data alone may not be sufficient to fully characterize complex physiological changes. There is still potential for further improvements in the study design of many exercise metabolomics studies. In particular, exercise-related parameters or measurements, such as exercise intensity and exercise duration, have a strong influence on metabolic changes after exercise training. Researchers should incorporate and quantify these parameters more consistently in study designs, which would facilitate comparisons between studies. Another important goal of exercise metabolomics research is to routinely use and integrate more histological (proteomics, genomics, transcriptomics) techniques in study design. Metabolomics should not be an "island," and the integration of multi-omics data will help researchers to further understand the interactions between genes, proteins, metabolites, and the environment, and to gain a deeper understanding of the effects of exercise on the organism.

Summary and outlook

Exercise metabolomics provides researchers in the field of exercise science with an effective research tool to search for potential biomarkers and therapeutic targets by detecting metabolite changes in a variety of biological fluids and tissues

after exercise in athletes and patients with chronic diseases, thus helping to improve the practical ability and depth of theoretical understanding of exercise performance and exercise prevention and treatment of chronic diseases.

With the continuous maturation of the technology and the deepening of the research, future exercise metabolomics research will further evaluate the exercise performance of outstanding athletes, so that their physiological conditions can be accurately assessed, which will provide a scientific basis for the development of precise training load programs for athletes and help coaches to cultivate outstanding athletes in a more effective way. In addition, mass spectrometry-based metabolomics testing is important for the treatment of metabolic disorders and provides clinicians with effective targets for the treatment of metabolic disorders, which is helpful for the treatment of chronic metabolic disorders. Meanwhile, mass spectrometry-based metabolomics studies will cover more subjects and will identify more metabolites. It is expected that more points of interest will emerge in the field of exercise metabolism and sports nutrition, focusing on the use of metabolomics findings to further design personalized and precise nutritional regimens to maximize the health benefits of physical performance and exercise. Finally, from the category of research groups, exercise metabolomics should focus more on human studies and more on practical orientated, reality-based experimental designs, collecting non-invasive sample collection methods based on urine and saliva. Such a trend will make exercise metabolomics research more informative and popular with participants, and lead to better and more accessible research tools for researchers, athletes, and coaches.

Author contributions

SQ: Writing–original draft. XL: Writing–review and editing. JY: Writing–review and editing. LY: Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This work was supported by a grant from the key program of Shandong Institute of Physical Education Research Start-up Funds (No. 2390015).

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.

Publisher's note

References

Al-Khelaifi, F., Diboun, I., Donati, F., Botrè, F., Alsayrafi, M., Georgakopoulos, C., et al. (2018). A pilot study comparing the metabolic profiles of elite-level athletes from different sporting disciplines. *Sports Med. Open* 4 (1), 2. doi:10.1186/s40798-017-0114-z

Babu, A. F., Csader, S., Männistö, V., Tauriainen, M. M., Pentikäinen, H., Savonen, K., et al. (2022). Effects of exercise on NAFLD using non-targeted metabolomics in adipose tissue, plasma, urine, and stool. *Sci. Rep.* 12 (1), 6485. doi:10.1038/s41598-022-10481-9

Belhaj, M. R., Lawler, N. G., and Hoffman, N. J. (2021). Metabolomics and lipidomics: expanding the molecular landscape of exercise biology. *Metabolites* 11 (3), 151–234. doi:10.3390/metabo11030151

Bester, R., Stander, Z., Mason, S., Keane, K. M., Howatson, G., Clifford, T., et al. (2021). Characterizing marathon-induced metabolic changes using (1)H-NMR metabolomics. *Metabolites* 11 (10), 656. doi:10.3390/metabo11100656

Bihlmeyer, N. A., Kwee, L. C., Clish, C. B., Deik, A. A., Gerszten, R. E., Pagidipati, N. J., et al. (2021). Metabolomic profiling identifies complex lipid species and amino acid analogues associated with response to weight loss interventions. *PLoS One* 16 (5), e0240764. doi:10.1371/journal.pone.0240764

Blackburn, G., Hay, J., Skagen, C., Paul, E., Achcar, F., Wilson, J., et al. (2020). Running on empty: a metabolomics approach to investigating changing energy metabolism during fasted exercise and rest. *Metabolites* 10 (10), 399. doi:10.3390/metabo10100399

Cai, M., Wu, C., Jing, C., Shen, X., He, M., Wang, L., et al. (2022). Blood metabolomics analysis identifies differential serum metabolites in elite and sub-elite swimmers. *Front. Physiol.* 13, 858869. doi:10.3389/fphys.2022.858869

Castro, A., Duft, R. G., Zeri, A. C. M., Cavaglieri, C. R., and Chacon-Mikahil, M. P. T. (2020). Commentary: metabolomics-based studies assessing exercise-induced alterations of the human metabolome: a systematic review. *Front. Physiol.* 11, 353. doi:10.3389/fphys.2020.00353

Contrepois, K., Wu, S., Moneghetti, K. J., Hornburg, D., Ahadi, S., Tsai, M. S., et al. (2020). Molecular choreography of acute exercise. *Cell* 181 (5), 1112–1130. doi:10.1016/j.cell.2020.04.043

Cronin, O., Barton, W., Skuse, P., Penney, N. C., Garcia-Perez, I., Murphy, E. F., et al. (2018). A prospective metagenomic and metabolomic analysis of the impact of exercise and/or whey protein supplementation on the gut microbiome of sedentary adults. *mSystems* 3 (3), e00044-18. doi:10.1128/mSystems.00044-18

Di Minno, A., Gelzo, M., Caterino, M., Costanzo, M., Ruoppolo, M., and Castaldo, G. (2022). Challenges in metabolomics-based tests, biomarkers revealed by metabolomic analysis, and the promise of the application of metabolomics in precision medicine. *Int. J. Mol. Sci.* 23 (9), 5213. doi:10.3390/ijms23095213

Enea, C., Seguin, F., Petitpas-Mulliez, J., Boildieu, N., Boisseau, N., Delpech, N., et al. (2010). (1)H NMR-based metabolomics approach for exploring urinary metabolome modifications after acute and chronic physical exercise. *Anal. Bioanal. Chem.* 396 (3), 1167–1176. doi:10.1007/s00216-009-3289-4

Febvey-Combes, O., Jobard, E., Rossary, A., Pialoux, V., Foucaut, A. M., Morelle, M., et al. (2021). Effects of an exercise and nutritional intervention on circulating biomarkers and metabolomic profiling during adjuvant treatment for localized breast cancer: results from the PASAPAS feasibility randomized controlled trial. *Integr. Cancer Ther.* 20, 1534735420977666. doi:10.1177/1534735420977666

Fiorenza, M., Hostrup, M., Gunnarsson, T. P., Shirai, Y., Schena, F., Iaia, F. M., et al. (2019). Neuromuscular fatigue and metabolism during high-intensity intermittent exercise. *Med. Sci. Sports Exerc* 51 (8), 1642–1652. doi:10.1249/mss.0000000000001959

Gehlert, S., Weinisch, P., Römisch-Margl, W., Jaspers, R. T., Artati, A., Adamski, J., et al. (2022). Effects of acute and chronic resistance exercise on the skeletal muscle metabolome. *Metabolites* 12 (5), 445. doi:10.3390/metabo12050445

Granacher, U., Lesinski, M., Büsch, D., Muehlbauer, T., Prieske, O., Puta, C., et al. (2016). Effects of resistance training in youth athletes on muscular fitness and athletic performance: a conceptual model for long-term athlete development. *Front. Physiol.* 7, 164. doi:10.3389/fphys.2016.00164

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

Høeg, T. B., Chmiel, K., Warrick, A. E., Taylor, S. L., and Weiss, R. H. (2020). Ultramarathon plasma metabolomics: phosphatidylcholine levels associated with running performance. *Sports (Basel)* 8 (4), 44. doi:10.3390/sports8040044

Hudson, J. F., Phelan, M. M., Owens, D. J., Morton, J. P., Close, G. L., and Stewart, C. E. (2021). Fuel for the damage induced": untargeted metabolomics in elite rugby union match play. *Metabolites* 11 (8), 544. doi:10.3390/metabo11080544

Kelly, R. S., Kelly, M. P., and Kelly, P. (2020). Metabolomics, physical activity, exercise and health: a review of the current evidence. *Biochim. Biophys. Acta Mol. Basis Dis.* 1866 (12), 165936. doi:10.1016/j.bbadis.2020.165936

Khoramipour, K., Sandbakk, Ø., Keshteli, A. H., Gaeini, A. A., Wishart, D. S., and Chamari, K. (2022). Metabolomics in exercise and sports: a systematic review. *Sports Med.* 52 (3), 547–583. doi:10.1007/s40279-021-01582-y

Kirwan, G. M., Coffey, V. G., Niere, J. O., Hawley, J. A., and Adams, M. J. (2009). Spectroscopic correlation analysis of NMR-based metabonomics in exercise science. *Anal. Chim. Acta* 652 (1-2), 173–179. doi:10.1016/j.aca.2009.07.011

Kistner, S., Döring, M., Krüger, R., Rist, M. J., Weinert, C. H., Bunzel, D., et al. (2021). Sex-specific relationship between the cardiorespiratory fitness and plasma metabolite patterns in healthy humans-results of the KarMeN study. *Metabolites* 11 (7), 463. doi:10.3390/metabo11070463

Klein, D. J., Anthony, T. G., and McKeever, K. H. (2021). Metabolomics in equine sport and exercise. *J. Anim. Physiol. Anim. Nutr. Berl.* 105 (1), 140–148. doi:10.1111/jpn.13384

Koay, Y. C., Stanton, K., Kienzle, V., Li, M., Yang, J., Celermajer, D. S., et al. (2021). Effect of chronic exercise in healthy young male adults: a metabolomic analysis. *Cardiovasc Res.* 117 (2), 613–622. doi:10.1093/cvr/cvaa051

Kozlowska, L., Mizera, O., and Mroz, A. (2020). An untargeted metabolomics approach to investigate the metabolic effect of beetroot juice supplementation in fencers-A preliminary study. *Metabolites* 10 (3), 100. doi:10.3390/metabo10030100

Krzysztofik, M., Wilk, M., Wojdała, G., and Gołaś, A. (2019). Maximizing muscle hypertrophy: a systematic review of advanced resistance training techniques and methods. *Int. J. Environ. Res. Public Health* 16 (24), 4897. doi:10.3390/ijerph16244897

Kujala, U. M., Mäkinen, V. P., Heinonen, I., Soininen, P., Kangas, A. J., Leskinen, T. H., et al. (2013). Long-term leisure-time physical activity and serum metabolome. *Circulation* 127 (3), 340–348. doi:10.1161/circulationaha.112.105551

Lemonakis, N., Mougios, V., Halabalaki, M., Dagla, I., Tsarbopoulos, A., Skaltsounis, A. L., et al. (2022). Effect of supplementation with olive leaf extract enriched with oleuropein on the metabolome and redox status of athletes' blood and urine-A metabolomic approach. *Metabolites* 12 (2), 195. doi:10.3390/metabo12020195

Liu, J., Zhu, L., Liao, J., and Liu, X. (2021). Effects of extreme weight loss on cardiometabolic health in children with metabolic syndrome: a metabolomic study. *Front. Physiol.* 12, 731762. doi:10.3389/fphys.2021.731762

Martins Conde, P., Pfau, T., Pires Pacheco, M., and Sauter, T. (2021). A dynamic multi-tissue model to study human metabolism. *NPJ Syst. Biol. Appl.* 7 (1), 5. doi:10.1038/s41540-020-00159-1

McClain, K. M., Friedenreich, C. M., Matthews, C. E., Sampson, J. N., Check, D. P., Brenner, D. R., et al. (2022). Body composition and metabolomics in the alberta physical activity and breast cancer prevention trial. *J. Nutr.* 152 (2), 419–428. doi:10.1093/jn/nxab388

Mendham, A. E., Goedecke, J. H., Zeng, Y., Larsen, S., George, C., Hauksson, J., et al. (2021). Exercise training improves mitochondrial respiration and is associated with an altered intramuscular phospholipid signature in women with obesity. *Diabetologia* 64 (7), 1642–1659. doi:10.1007/s00125-021-05430-6

Monnerat, G., Sánchez, C. A. R., Santos, C. G. M., Paulucio, D., Velasque, R., Evaristo, G. P. C., et al. (2020). Different signatures of high cardiorespiratory capacity revealed with metabolomic profiling in elite athletes. *Int. J. Sports Physiol. Perform.* 15, 1156–1167. doi:10.1123/ijspp.2019-0267

Moreira, L. P., Silveira, L., Jr., Pacheco, M. T. T., da Silva, A. G., and Rocco, D. (2018). Detecting urine metabolites related to training performance in swimming athletes by means of Raman spectroscopy and principal component analysis. *J. Photochem Photobiol. B* 185, 223–234. doi:10.1016/j.jphotobiol.2018.06.013

Nayor, M., Chernofsky, A., Miller, P. E., Spartano, N. L., Murthy, V. L., Malhotra, R., et al. (2022). Integrative analysis of circulating metabolite levels that correlate with physical activity and cardiorespiratory fitness. *Circ. Genom Precis. Med.* 15 (3), e003592. doi:10.1161/circgen.121.003592

Nicolaides, N. C., Ioannidi, M. K., Koniari, E., Papageorgiou, I., Bartzeliotou, A., Sertedaki, A., et al. (2021). Untargeted plasma metabolomics unravels a metabolic signature for tissue sensitivity to glucocorticoids in healthy subjects: its implications in dietary planning for a healthy lifestyle. *Nutrients* 13 (6), 2120. doi:10.3390/nu13062120

Osawa, S., Kato, H., Maeda, Y., Takakura, H., Ogasawara, J., and Izawa, T. (2021). Metabolomic profiles in adipocytes differentiated from adipose-derived stem cells following exercise training or high-fat diet. *Int. J. Mol. Sci.* 22 (2), 966. doi:10.3390/ijms22020966

Palmnäs, M. S. A., Kopciuk, K. A., Shaykhutdinov, R. A., Robson, P. J., Mignault, D., Rabasa-Lhoret, R., et al. (2018). Serum metabolomics of activity energy expenditure and its relation to metabolic syndrome and obesity. *Sci. Rep.* 8 (1), 3308. doi:10.1038/s41598-018-21585-6

Pellegrino, J. K., Anthony, T. G., Gillies, P., and Arent, S. M. (2022). The exercise metabolome: acute aerobic and anaerobic signatures. *J. Int. Soc. Sports Nutr.* 19 (1), 603–622. doi:10.1080/15502783.2022.2115858

Pohjanen, E., Thysell, E., Jonsson, P., Eklund, C., Silfver, A., Carlsson, I. B., et al. (2007). A multivariate screening strategy for investigating metabolic effects of strenuous physical exercise in human serum. *J. Proteome Res.* 6 (6), 2113–2120. doi:10.1021/pr070007g

Pugh, J. N., Phelan, M. M., Caamaño-Gutiérrez, E., Sparks, S. A., Morton, J. P., Close, G. L., et al. (2021). Four weeks of probiotic supplementation alters the metabolic perturbations induced by marathon running: insight from metabolomics. *Metabolites* 11 (8), 535. doi:10.3390/metabo11080535

Qiu, S., Cai, Y., Yao, H., Lin, C., Xie, Y., Tang, S., et al. (2023). Small molecule metabolites: discovery of biomarkers and therapeutic targets. *Signal Transduct. Target Ther.* 8 (1), 132. doi:10.1038/s41392-023-01399-3

Remie, C. M. E., Janssens, G. E., Bilet, L., van Weeghel, M., Duvivier, B., de Wit, V. H. W., et al. (2021). Sitting less elicits metabolic responses similar to exercise and enhances insulin sensitivity in postmenopausal women. *Diabetologia* 64 (12), 2817–2828. doi:10.1007/s00125-021-05558-5

Rivera-Brown, A. M., and Frontera, W. R. (2012). Principles of exercise physiology: responses to acute exercise and long-term adaptations to training. *Pm R.* 4 (11), 797–804. doi:10.1016/j.pmrj.2012.10.007

Sakaguchi, C. A., Nieman, D. C., Signini, E. F., Abreu, R. M., and Catai, A. M. (2019). Metabolomics-based studies assessing exercise-induced alterations of the human metabolome: a systematic review. *Metabolites* 9 (8), 164. doi:10.3390/metabo9080164

San-Millán, I., Stefanoni, D., Martinez, J. L., Hansen, K. C., D'Alessandro, A., and Nemkov, T. (2020). Metabolomics of endurance capacity in world tour professional cyclists. *Front. Physiol.* 11, 578. doi:10.3389/fphys.2020.00578

Schader, J. F., Haid, M., Cecil, A., Schoenfeld, J., Halle, M., Pfeufer, A., et al. (2020). Metabolite shifts induced by marathon race competition differ between athletes based on level of fitness and performance: a substudy of the enzy-MagIC study. *Metabolites* 10 (3), 87. doi:10.3390/metabo10030087

Schranner, D., Kastenmüller, G., Schönfelder, M., Römisch-Margl, W., and Wackerhage, H. (2020). Metabolite concentration changes in humans after a bout of exercise: a systematic review of exercise metabolomics studies. *Sports Med. Open* 6 (1), 11. doi:10.1186/s40798-020-0238-4

Schranner, D., Schönfelder, M., Römisch-Margl, W., Scherr, J., Schlegel, J., Zelger, O., et al. (2021). Physiological extremes of the human blood metabolome: a metabolomics analysis of highly glycolytic, oxidative, and anabolic athletes. *Physiol. Rep.* 9 (12), e14885. doi:10.14814/phy2.14885

Shen, C. L., Watkins, B. A., Kahathuduwa, C., Chyu, M. C., Zabet-Moghaddam, M., Elmassry, M. M., et al. (2021). Tai chi improves brain functional connectivity and plasma lysophosphatidylcholines in postmenopausal women with knee osteoarthritis: an exploratory pilot study. *Front. Med. (Lausanne)* 8, 775344. doi:10.3389/fmed.2021.775344

Shi, M., Ellingsen, Ø., Bathen, T. F., Høydal, M. A., Stølen, T., and Esmaeili, M. (2019). The effect of exercise training on myocardial and skeletal muscle metabolism by MR spectroscopy in rats with heart failure. *Metabolites* 9 (3), 53. doi:10.3390/metabo9030053

Shimada, Y. J., Batra, J., Kochav, S. M., Patel, P., Jung, J., Maurer, M. S., et al. (2021). Difference in metabolomic response to exercise between patients with and without hypertrophic cardiomyopathy. *J. Cardiovasc Transl. Res.* 14 (2), 246–255. doi:10.1007/s12265-020-10051-2

Siopi, A., Deda, O., Manou, V., Kosmidis, I., Komninou, D., Raikos, N., et al. (2019). Comparison of the serum metabolic fingerprint of different exercise modes in men with and without metabolic syndrome. *Metabolites* 9 (6), 116. doi:10.3390/metabo9060116

Tabone, M., Bressa, C., García-Merino, J. A., Moreno-Pérez, D., Van, E. C., Castelli, F. A., et al. (2021). The effect of acute moderate-intensity exercise on the serum and fecal metabolomes and the gut microbiota of cross-country endurance athletes. *Sci. Rep.* 11 (1), 3558. doi:10.1038/s41598-021-82947-1

Tokarz, J., Möller, G., Artati, A., Huber, S., Zeigerer, A., Blaauw, B., et al. (2021). Common muscle metabolic signatures highlight arginine and lysine metabolism as potential therapeutic targets to combat unhealthy aging. *Int. J. Mol. Sci.* 22 (15), 7958. doi:10.3390/ijms22157958

Tzimou, A., Benaki, D., Nikolaidis, S., Mikros, E., Taitzoglou, I., and Mougios, V. (2020). Effects of lifelong exercise and aging on the blood metabolic fingerprint of rats. *Biogerontology* 21 (5), 577–591. doi:10.1007/s10522-020-09871-1

Wishart, D. S., Li, C., Marcu, A., Badran, H., Pon, A., Budinski, Z., et al. (2020). PathBank: a comprehensive pathway database for model organisms. *Nucleic Acids Res.* 48 (D1), D470–d478. doi:10.1093/nar/gkz861

Yan, B., Liu, Y., Shi, A., Wang, Z., Aa, J., Huang, X., et al. (2018). Investigation of the antifatigue effects of Korean ginseng on professional athletes by gas chromatography-time-of-flight-mass spectrometry-based metabolomics. *J. AOAC Int.* 101 (3), 701–707. doi:10.5740/jaoacint.17-0220

Zhang, X., Wang, X., Le, S., Ojanen, X., Tan, X., Wiklund, P., et al. (2020). Effects of exercise and dietary interventions on serum metabolites in men with insomnia symptoms: a 6-month randomized controlled trial. *Sports Med. Health Sci.* 2 (2), 95–101. doi:10.1016/j.smhs.2020.05.002

Zhou, W., Zeng, G., Lyu, C., Kou, F., Zhang, S., and Wei, H. (2021). The effect of strength-endurance training on serum and urine metabolic profiles of female adolescent volleyball athletes. *Physiol. Int.* 108 (2), 285–302. doi:10.1556/2060.2021.00150

frontiers | Frontiers in Physiology

TYPE Mini Review
PUBLISHED 16 January 2024
DOI 10.3389/fphys.2024.1309663

OPEN ACCESS

EDITED BY
Tania Gamberi,
University of Florence, Italy

REVIEWED BY
Chantal Verkindt,
Université de la Réunion, France

*CORRESPONDENCE
Xinxin Zhang,
zhangxx9606@snnu.edu.cn
Weiguo Liu,
liuwg@mailbox.gxnu.edu.cn

†These authors have contributed equally to this work and share first authorship

RECEIVED 08 October 2023
ACCEPTED 04 January 2024
PUBLISHED 16 January 2024

CITATION
Wang Z, Liu X, Gao K, Tuo H, Zhang X and Liu W (2024), Clinical effects and biological mechanisms of exercise on lumbar disc herniation.
Front. Physiol. 15:1309663.
doi: 10.3389/fphys.2024.1309663

Clinical effects and biological mechanisms of exercise on lumbar disc herniation

Ziwen Wang[1†], Xindai Liu[2†], Ke Gao[1], Haowen Tuo[1], Xinxin Zhang[1*] and Weiguo Liu[1*]

[1]College of Physical Education and Health, Guangxi Normal University, Guilin, China, [2]College of International Culture and Education, Guangxi Normal University, Guilin, China

Lumbar Disc Herniation (LDH) is a syndrome in which lumbar disc degeneration, rupture of the annulus fibrosus, and herniation of the nucleus pulposus irritate and compress the nerve roots and cauda equina, resulting in the main manifestations of lumbar pain and/or lower extremity pain. There is evidence in various clinical areas that exercise is effective in treating LDH, and exercise intervention for more than 2 weeks reduces disease activity in LDH. However, the mechanism of exercise's action in reducing disease activity in LDH is unclear. In this article, we first summarize and highlight the effectiveness of exercise in treating LDH and provide guideline recommendations regarding exercise type, intensity, frequency, and duration. Then, we integrate the existing evidence and propose biological mechanisms for the potential effects of exercise on neuromechanical compression, inflammatory chemical stimuli, and autoimmune responses from the perspective of LDH pathogenesis as an entry point. However, a large body of evidence was obtained from non-LDH populations. Future research needs to investigate further the proposed biological mechanisms of exercise in reducing disease activity in LDH populations. This knowledge will contribute to the basic science and strengthen the scientific basis for prescribing exercise therapy for the routine clinical treatment of LDH.

KEYWORDS

exercise, lumbar disc herniation, clinical effect, biological mechanisms, review

1 Introduction

Lumbar Disc Herniation (LDH) is a syndrome in which lumbar disc degeneration, rupture of the annulus fibrosus, and protrusion of the nucleus pulposus irritate and compress the nerve root and cauda equina, resulting in lumbar pain and/or lower limb pain (Zheng et al., 2021). In recent years, the incidence of LDH has been increasing year by year, and the incidence among young people has been rising due to the long-term use of sedentary postures for study and work and the reduction of physical activity (Frino et al., 2006). LDH has caused severe impacts on the daily life and work of patients, and the increasing incidence of LDH has caused an enormous burden on society (Katz, 2006). Therefore, the search for a safe, effective, and generalizable method for preventing and treating LDH is a social problem that needs to be solved by medical practitioners today.

The therapeutic mechanism of LDH is closely related to the recovery of low back muscle function. As the slow muscle fibres of the paravertebral muscles are significantly reduced in patients with low back pain, their role in maintaining trunk posture and body position is

TABLE 1 Characteristics of included studies.

Study		LDH patient	Exercise						Clinical effects					
Author, year	Design	N, age	Intervention group (IG)			Control group (CG)			JOA	ODI	VAS	ROM	NHP or QOL	Effectiveness of the intervention
			Type	Frequency and Duration	Time	Type	Frequency and Duration	Time						
Taşpınar G, et al. (Taşpınar et al., 2023)	RCT	54, 50.3 ± 6.7	Pilates exercise therapy	6 weeks × 3 sessions	45–60 min	Routines without doing any exercises	6 weeks × 3 sessions × 30 min	45–60 min		↑	↑		↑	**After 6 weeks of exercise** Joint pain (VAS), functional disability (ODI), and degree of improvement in mood depression (QOL) were significantly improved. (IG vs CG)
Selim M N, et al. (Selim et al., 2022)	RCT	15, 48.5 ± 5.8	A.Mulligan spinal mobilization with leg movement and transcutaneous electrical nerve stimulation B. McKenzie and transcutaneous electrical nerve stimulation	4 weeks × 3 sessions	NR	Transcutaneous electrical nerve stimulation	4 weeks × 3 sessions ×	30 min		↑	↑			**After 4 weeks of exercise** Pain conditions (VAS) and functional status (ODI) were significantly improved. (IG vs CG)
Deniz Bayraktar et al. (Bayraktar et al., 2016)	RCT	31, 41.5 ± 23.5	Water specific therapy	8 weeks × 3 sessions	60 min	Bridging, trunk-curl, quadrupedal, side lying, sitting on a ball and standing	8 weeks × 3 sessions	60 min		—	—		—	After 4 weeks of exercise Pain conditions (VAS), functional status (ODI) and degree of improvement in mood depression (NHP) had no significant effect
Shen zhixiang, et al. (Shen et al., 2009)	RCT	30, 47.2 ± 11.7	Swiss ball exercises and lumbar traction	4 weeks × 6 sessions	30 min	Lumbar traction	NR	NR			↑			**After 4 weeks of exercise** Joint pain (VAS) were significantly improved. (IG vs CG)
Yildirim P, et al. (Yildirim and Gultekin., 2022)	RCT	48, 37.9 ± 7.5	Yoga exercise and patient education	12 weeks × 2 sessions	60 min	Patient education and routines without doing any exercises	12 weeks × 2 sessions	60 min		↑	↑			**After 12 weeks of exercise** Pain conditions (VAS), and functional status (ODI) were significantly improved. (IG vs CG)

(Continued on following page)

10.3389/fphys.2024.1309663

TABLE 1 (*Continued*) Characteristics of included studies.

Study		LDH patient	Exercise						Clinical effects					
Author, year	Design	N, age	Intervention group (IG)			Control group (CG)			JOA	ODI	VAS	ROM	NHP or QOL	Effectiveness of the intervention
			Type	Frequency and Duration	Time	Type	Frequency and Duration	Time						
Iosub, Monica Elena, et al. (Iosub et al., 2023)	RCT	77, 50. ± 13.1	Vojta therapy and procedures, mobility, strength exercises and motor control exercise	2 weeks × 5 sessions	80 min	Mobility and strength exercises and motor control exercises	2 weeks × 5 sessions	50 min		↑	↑		↑	**After 2 weeks of exercise** Pain conditions (VAS), disability level, mobility (ODI), strength, and health-related quality of life (NHP) were significantly improved. (IG)
Gulsen, Mustafa, al. (Gulsen et al., 2019)	RCT	64, 53.0 ± 14.6	A.Lumbar stabilization training B.proprioceptive neuromuscular facilitation C.physical therapy	4 weeks × 5 sessions	45 min	Without any application	NR	NR		↑	↑			**After 2 weeks of exercise** Muscle strength and endurance in the lumbar, pain conditions (VAS), and functional status (ODI) improved significantly. (IG vs. CG)
Xu, J. et al. (Xu et al., 2020)	RCT	72, NR	Shi-style spine balance manipulation combined with Daoyin therapy	4 weeks × 3 sessions	20 min	Lumbar mechanical traction	4 weeks × 3 sessions	20 min		↑	↑			**After 4 weeks of exercise** Pain conditions (VAS) and comfort level (ODI) were significantly improved. (IG vs. CG)
Zhou Xin et al. (Zhou et al., 2022)	RCT	270, 40.0 ± 20.0	Traditional Chinese exercise combined with massage	6 weeks × 3 sessions	30 min	Traditional Chinese massage	6 weeks × 3 sessions	30 min		↑	↑	↑		**After 6 weeks of exercise** Pain conditions (VAS), functional status (ODI), and lumbar spine activities (ROM) were significantly improved. (IG vs CG)
Khanzadeh, R. et al. (Khanzadeh et al., 2020)	RCT	30, 40.3 ± 7.7	Suspension core stability exercises	8 weeks × 3 sessions	60 min	Conventional core stability exercises	8 weeks × 3 sessions	30 min			↑			**After 8 weeks of exercise** Joint pain (VAS) was significantly improved. (IG vs CG)
França F R et al. (França et al., 2013)	RCT	23, 45.1 ± 6.3	Exercises of lumbar segmental stabilization	8 weeks × 2 sessions	60 min	Electrotherapy	8 weeks × 2 sessions ×	60 min		↑	↑			**After 8 weeks of exercise** Joint pain (VAS) and functional status (ODI) were significantly improved. (IG vs CG)

(Continued on following page)

10.3389/fphys.2024.1309663

TABLE 1 (*Continued*) Characteristics of included studies.

Study		LDH patient	Exercise						Clinical effects					
Author, year	Design	N, age	Intervention group (IG)			Control group (CG)			JOA	ODI	VAS	ROM	NHP or QOL	Effectiveness of the intervention
			Type	Frequency and Duration	Time	Type	Frequency and Duration	Time						
Javaheri A H (Javaheri et al., 2011)	RCT	30, 41.6 ± 5.0	Exercise Therapy and Massage	8 weeks × 3 sessions	60 min	No special activity	8 weeks × 3 sessions	60 min					↑	**After 8 weeks of exercise** Quality of life (QOL) improved significantly. (IG vs CG)
Lu Weiwei et al. (LU et al., 2014)	RCT	50, 43.8 ± 5.6	Physiotherapy core stability exercise and Proprioceptive training	8 weeks × 6 sessions	35 min	Physiotherapy and core stability exercise	8 weeks × 6 sessions	15 min		↑	↑			**After 6 weeks of exercise** Pain conditions (VAS), muscle strength, and comfort level (ODI) were significantly improved. (IG vs CG)
Dae-Keun Jeong et al. (Jeong et al., 2017)	RCT	30, 33.3 ± 9.4	Balance center stabilization resistance exercise	4 weeks × 3 sessions	30 min	three-dimensional stabilization exercise group	4 weeks × 3 sessions	30 min		↑				**After 4 weeks of exercise** Pain conditions and muscle strength (ODI) were significantly improved. (IG)
Liu Ming et al. (Liu and Gao., 2020)	RCT	120, 38.72 ± 2.37	Conventional traction therapy and Mulligan technique and Swiss ball exercises	2 weeks × 7 sessions	60 min	Conventional traction therapy	2 weeks × 7 sessions	30 min	↑		↑			**After 2 weeks of exercise** Pain conditions (VAS), Restoration of functionality (JOA), and living conditions were significantly improved. (IG vs CG)

Notes: RCT, randomized controlled trial; JOA, japanese orthopedic association; ODI, oswestry disability index; VAS, visual analog scales; NHP, nottingham health profile; QOL, quality of life; ROM, range of motion; ↑ Indicate clinical effects improved; — Indicate no significant effect; NR, not reported. Bold values represents the duration of the intervention.

10.3389/fphys.2024.1309663

weakened, and it is easy to have lumbar back muscle fatigue (Mayer et al., 1985). Prolonged lumbar back muscle fatigue will lead to dysfunction of the tissue structure that maintains the endogenous and exogenous stability of the lumbar spine, thus gradually losing the function of maintaining the spine's stability, leading to or aggravating LDH (Stevens et al., 2007). Despite the availability of several surgical and non-surgical measures for treating LDH (Loupasis et al., 1999), previous studies have shown that 85% of patients prefer to be treated in non-surgical treatment (Kreiner et al., 2014). This may be because the two treatments have no significant difference in therapeutic efficacy (Yorimitsu et al., 2001). More importantly, non-surgical conservative treatment measures dominated by exercise therapy are highly beneficial in enhancing the function of lower back muscles (McGill, 1998) The 2007 clinical practice guideline "Diagnosis and treatment of lower back pain," jointly published by the American College of Physicians and the Pain Society, states that there is moderately strong evidence that exercise therapy is effective in the treatment of chronic low back pain (Chou et al., 2007); The United Kingdom National Institute for Health and Care Excellence guideline issued in 2010 added a recommendation for conservative treatment as a first-line treatment and stated that there is strong evidence that exercise therapy is effective in the treatment of chronic low back pain (Bernstein et al., 2017); and continued reports have shown that targeted selection of exercise exercises can enhance low back muscle function and reduce their fatigue (Merritt and Merritt, 2007; Kim et al., 2013; Lee et al., 2013; Gürşen et al., 2016; Sipaviciene and Kliziene, 2020; Li, 2021).

The clinical effects of exercise on LDH have been extensively studied. However, to the best of our knowledge, the biological mechanisms of how exercise promotes recovery from LDH have not yet been fully explored. Exploring the underlying biological mechanisms is essential for understanding the pathogenesis of LDH and proposing new exercise therapies.

2 Clinical effects of exercise on patients with LDH

To summarize the evidence for the clinical effects of exercise in LDH, we conducted a literature search on PubMed and Google Scholar using keywords (exercise, physical activity, clinical effects, and lumbar disc herniation) to identify relevant trials and review articles. Because there were overlapping trial and review articles, and some review articles had a specific focus (e.g., a particular type of exercise or clinical area). This article only reviews the 15 randomized controlled trial (RCT) studies published between 2009 and 2023 from the search results. The characteristics of the included articles and the effects of the exercise intervention are shown in Table 1.

Exercise has a multifaceted positive impact on the clinical outcome of LDH. Firstly, exercise could effectively reduce pain (Shen et al., 2009; França et al., 2013; Gulsen et al., 2019; Khanzadeh et al., 2020; Xu et al., 2020; Selim et al., 2022; Yildirim and Gultekin, 2022; Zhou et al., 2022; Iosub et al., 2023; Taşpınar et al., 2023), improve lumbar spine motion limitation (Zhou et al., 2022; Iosub et al., 2023), and significantly increase lumbar spine range of motion during forward flexion and backward extension. Secondly, exercise could be effective in improving quality of life, mental health, or sleep status (Javaheri et al., 2011; Taşpınar et al., 2023) so that patients could maintain a positive and sunny attitude towards the disease without exacerbating the severity of the disease activity or particular symptoms. In addition, some specific exercises could enhance muscle coordination, flexibility, and balance, thus improving the stability of the lumbar spine (França et al., 2013; LU et al., 2014; Gulsen et al., 2019). In sum, exercise interventions positively affect rehabilitation and overall health in patients with LDH.

As shown in Table 1, the effectiveness of exercise interventions may be related to types, duration, intensity, and frequency. Regarding exercise types, this study found that the included studies were all based on non-acute self-weighted exercises for the lumbar and back core muscles and that different types of exercises had different effects on clinical effectiveness. Specifically, Mulligan spinal mobilization with leg movement was more effective than the McKenzie method (Selim et al., 2022), lumbar stabilization training was more effective than proprioceptive neuromuscular facilitation (LU et al., 2014), and suspension core stability training was more effective than traditional core training (Khanzadeh et al., 2020). However, current research lacks comparative studies of the effects of multiple exercise-type interventions, so the optimal type of exercise is unclear. Regarding exercise duration, previous studies have shown that exercise training for 2–12 weeks or longer significantly improves physical performance and reduces the clinical severity of disease in patients with LDH (Yildirim and Gultekin, 2022). However, a 1-week exercise intervention was ineffective in patients' disease recovery (Li et al., 2022). Therefore, concerning the results of the current studies, exercise interventions in patients with LDH should be at least 2 weeks to enhance physical performance and reduce disease severity significantly. Regarding exercise intensity, the included studies did not finely classify exercise intensity. However, previous studies have shown that high-intensity strength training (1RM $\geq$ 70%) and aerobic training (maximal heart rate or maximal oxygen consumption $\geq$70%) have a negative effect on some elderly patients with LDH, producing symptoms such as joint damage (Saal, 1996). Therefore, the intensity of training should be rationally arranged according to the patient's condition when providing exercise interventions for patients, and large-intensity exercise interventions should be avoided as much as possible. In terms of exercise frequency, Kim et al. (Kim et al., 2010) found that the follow-up exercise intervention in 40 patients with LDH found that the patients two times/a week had significantly increased their lumbar strength, decreased their Oswestry dysfunction index and significantly decreased their back pain and leg pain scores. The patients who had one time/2 weeks and those who did not train had significantly decreased their lumbar strength, suggesting that the frequency of exercise should be kept at least two times/a week in order to have a significant effect.

In conclusion, exercise could improve physical performance, reduce pain, improve quality of life, improve mental health and sleep, and relieve lumbar fatigue in patients with LDH. However, it is necessary to investigate further what types of exercise, as well as the duration, frequency, and intensity of exercise, are most effective in treating LDH in the future.

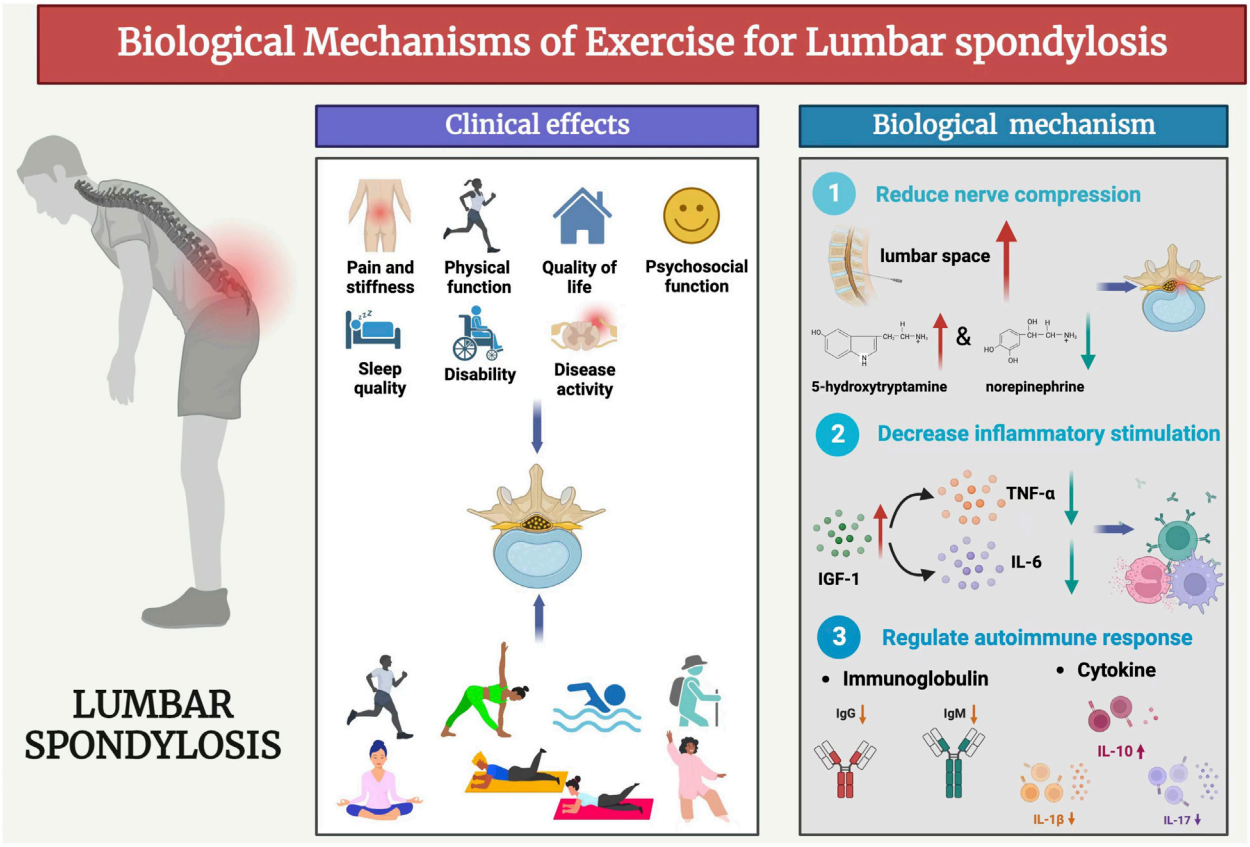


FIGURE 1
Biological mechanisms of exercise for lumbar spondylosis.

3 Biological mechanisms of exercise in the treatment of LDH

In recent years, the pathogenesis, diagnosis and treatment of LDH have been gradually improved through many experimental and clinical studies. Previous studies have concluded that the biological mechanisms of LDH pathogenesis are the mechanical compression doctrine, inflammatory chemical stimulation doctrine, and autoimmune doctrine (Meng et al., 2022). Exercise could produce a series of biological responses to the three doctrines and promote the recovery of LDH (Figure 1).

3.1 Biological mechanisms of exercise to reduce mechanical compression of nerves

The previous study found that taijiquan exercise positively affected LDH (Deng and Xia, 2018), and both peroneal motor and sensory nerves were significantly improved (Zou et al., 2019). This suggests that tai chi exercise improves the stability of the lumbosacral spine, relieves the compressed lumbosacral nerves to varying degrees, and then improves the conduction function of the peripheral nerves. In addition, previous studies have also shown that exercise significantly increased the metabolic level of the central nervous system mediator 5-hydroxytryptamine (Wipfli et al., 2011) while decreasing the sympathetic mediator norepinephrine in the vegetative nerves (Jia et al., 2020). This means that the central and vegetative functions are adjusted. Interestingly, the results of previous studies also found that herniated discs often do not directly compress the nerve roots but rather cause compression and congestion of the vertebral veins below the intervertebral foramina, limiting reflux, followed by impaired capillary blood flow and finally affecting arterial blood supply (Rydevik et al., 1984; Hoyland et al., 1989). Therefore, vertebral venous stasis is important in radicular pain (Parke and Watanabe, 1985). In this regard, long-term systematic exercise can produce well-adapted changes in human blood viscosity by appropriately decreasing it within a certain range (Pichon et al., 2016).

Based on the above studies, exercise could improve the function of the peripheral tissues of the spine, increase the lumbar spinal space, reduce and release the extrusion of the protruding material on the intervertebral foraminal nerve roots and vertebral organs, prompt the intervertebral disc space to generate negative pressure and reduce the pressure within the lumbar spinal interspace, and effectively improve the microcirculation of the lesion blocking localization (Lam et al., 2018). This may be an important biological mechanism for exercise to reduce the mechanical compression of nerves. However, there are no studies to investigate the effect of exercise on vertebral vein blood viscosity and other blood characteristics in LDH currently, and further research is needed.

3.2 Biological mechanisms by which exercise ameliorates inflammatory chemical stimuli

The innate immune system triggers inflammation once immune cells detect infection or tissue damage (Weyand and Goronzy, 2021). Previous studies have pointed out that inflammatory mediators and related cytokines are essential in LDH (Djuric et al., 2019), with tumour necrosis factor-alpha (TNF-α) being a key mediator of the inflammatory response (Driscoll et al., 1997). In addition, Pelosi et al. (Pelosi et al., 2007) found that localized expression of IGF-1 in skeletal muscle by transgenic techniques significantly downregulated the expression of the inflammatory factor TNF-α. The study of Wang et al. further demonstrated that the serum levels of inflammatory factors (such as IL-6 and TNF-α) were negatively correlated with the levels of IGF-1 (Wang et al., 2019). Therefore, IGF-1 has an inhibitory effect on inflammatory factors such as TNF-α and IL-6.

On the other hand, previous studies on exercise interventions have shown that exercise could affect IGF-1 levels in skeletal muscle and the circulatory system (Kim et al., 2019; Norling et al., 2020). IGFBP and IGF-1 levels in skeletal muscle were significantly upregulated in humans after high-intensity aerobic exercise (Kraemer et al., 2017). Resistance training also raises circulation IGF-1 levels (Rojas Vega et al., 2010), with intermittent aerobic exercise being more effective than continuous aerobic exercise (Żebrowska et al., 2018). The above studies have amply demonstrated the facilitating effect of exercise on IGF-1 synthesis, which may be an important biological mechanism by which exercise ameliorates inflammatory chemical stimuli. However, the dependence of IGF-1 on the type, duration, intensity, and frequency of exercise is not clear, so it is of great research value to explore the correlation in the future.

3.3 Biological mechanisms by which exercise modulates the autoimmune response

Few studies have investigated how exercise improves LDH by modulating the immune system. However, the mechanisms of the immune system's action on LDH have been discussed in various studies, mainly focusing on cytokine and immunoglobulins (Naylor et al., 1975; Miyamoto et al., 2000; Shamji et al., 2010). Regarding immune factors, previous studies (Miyamoto et al., 2000; Shamji et al., 2010; Al-Obaidi and Mahmoud, 2014; Djuric et al., 2020) detected the presence of large amounts of IL-1β, IL-17, and IL-10 in the intervertebral discs of patients with LDH and investigated the mechanism of their action on LDH. The results showed a significant negative correlation between the concentrations of IL-1β and IL-17 and the condition of LDH (Al-Obaidi and Mahmoud, 2014; Tan et al., 2022). At the same time, IL-10, a critical immunosuppressive factor, had a significant positive correlation with the condition of LDH (Uçeyler et al., 2007). Although fewer studies investigate the effects of exercise on modulating cytokine in patients with LDH, many previous studies have been conducted in normal populations. Faelli et al. (Faelli et al., 2020) found that IL-1β levels were significantly reduced after 24 sessions of HIFT training. Hoffman-Goetz et al. (Hoffman-Goetz et al., 2010) found that prolonged moderate to moderate-intensity exercise increased IL-10 secretion. In a study by Rahimi et al. (Rahimi and Hormones, 2019), 8 weeks of resistance training reduces IL-17 levels. Regular exercise (Alizadeh et al., 2015; Conroy et al., 2016; Karstoft and Pedersen, 2016) reduces IL-1β, IL-17 and increases IL-10 levels. Interestingly, some studies pointed out that the effect of exercise on cytokine may be related to the intensity and type of exercise, as Peake et al. (Peake et al., 2005) found that, after athletes ran at different exercise intensities, IL-10 was significantly increased in the high-intensity group, while there was no change in the other groups. IL-17 levels increased after high-intensity running but decreased after free exercise (Duzova et al., 2009; Cook et al., 2013). Based on the above findings, the present study concluded that exercise may improve LDH disease by decreasing the concentrations of IL-1β and IL-6 and increasing the concentration of IL-10. However, the improvement effect may be limited by the intensity or type of exercise, which could be further explored in future studies.

Regarding immunoglobulins, previous studies (Naylor et al., 1975; Kang et al., 1996; Duzova et al., 2009) demonstrated that the levels of IgG and IgM were significantly elevated in patients with LDH, and the concentrations of IgG and IgM were positively correlated with the severity of LDH. As with cytokine, few previous studies have investigated the effects of exercise on modulating immunoglobulins in patients with LDH, mainly focusing on studies in normal populations. Previous studies have shown that prolonged high-intensity exercise training reduces IgG, IgM, and IgA concentrations and increases the degree of reduction with increasing exercise load (Coppola et al., 2005). Interestingly, however, a study by Mitchell et al. (Mitchell et al., 1996) showed no significant change in IgG and IgM concentrations after 12 weeks of moderate-intensity exercise. In contrast, Nieman et al. (Nieman and Pedersen, 1999) found a significant increase in IgM and IgG concentrations after prolonged moderate-intensity exercise. This could be a difference caused by the different types of exercise in the two studies. To address the controversial phenomena in the existing studies, future studies should use randomized controlled trials to investigate the effects of different types or intensities of exercise on the immunoglobulins of patients with LDH and also to investigate further how other immunologically active substances act on patients with LDH, in order to find the optimal exercise therapy.

4 Conclusion

Extensive research has focused on the clinical efficacy of exercise in treating LDH. Substantial evidence indicates that exercise therapy's varying types, durations, and intensities are clinically effective for LDH, and in particular, that the use of non-acute self-weighted exercise types, exercise durations exceeding 2 weeks, and non-high-intensity exercise therapies could alleviate disease activity. Nevertheless, most current literature primarily emphasizes clinical observation. It relies on subjective scoring criteria like VAS

and JOA in its assessment, lacking quantitative and precise observation indexes to improve its credibility. Consequently, this review begins by examining the pathogenesis of LDH. It proceeds to delve into three facets of the biological mechanisms influenced by exercise: mechanical compression, inflammatory chemical stimulation, and autoimmunity. Subsequently, this review takes the pathogenesis of LDH as an entry point to discuss the biological mechanisms of exercise in three aspects: mechanical compression, inflammatory chemical stimulation, and autoimmunity. However, the relevant evidence is mainly based on non-LDH patients, and the effect of exercise type on the biological response of LDH patients is not yet clear and needs to be further explored.

5 Future directions

In response to the existing studies, we suggest that the biological response to exercise should be explored in patients with LDH, and we recommend a long intervention follow-up study. Future research directions can be explored in the following three areas: 1) The effect of exercise on vertebral venous blood viscosity and exploring adaptive changes; 2) Exercise plays a positive role in IGF-1 production, exploring the dependence of IGF-1 on exercise mode and intensity and the inhibitory effect of IGF-1 on the inflammatory factor THF-α; 3) Depending on the intensity and type of exercise, the effects of exercise on other immunoreactive substances were further investigated to reveal the potential modulatory effects of exercise on the immune system of patients with LDH and to determine the optimal exercise treatment measures.

Author contributions

ZW: Writing–original draft. XL: Visualization, Writing–original draft. KG: Visualization, Writing–original draft. HT: Visualization, Writing–original draft. XZ: Writing–review and editing. WL: Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by Guangxi Normal University Cross-disciplinary projects (2021JC011).

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.

Publisher's note

References

Alizadeh, H., Daryanoosh, F., Moatari, M., and Hoseinzadeh, K. J. M. j.o.t.I. R. o.I. (2015). Effects of aerobic and anaerobic training programs together with omega-3 supplement on interleukin-17 and CRP plasma levels in male mice. *Med. J. Islam. Repub. Iran.* 29, 236.

Al-Obaidi, S., and Mahmoud, F. (2014). Immune responses following McKenzie lumbar spine exercise in individuals with acute low back pain: a preliminary study. *Acta Med. Acad.* 43 (1), 19–29. doi:10.5644/ama2006-124.96

Bayraktar, D., Guclu-Gunduz, A., Lambeck, J., Yazici, G., Aykol, S., and Demirci, H. (2016). A comparison of water-based and land-based core stability exercises in patients with lumbar disc herniation: a pilot study. *Disabil. Rehabil.* 38 (12), 1163–1171. doi:10.3109/09638288.2015.1075608

Bernstein, I. A., Malik, Q., Carville, S., and Ward, S. (2017). Low back pain and sciatica: summary of NICE guidance. *Bmj* 356, i6748. doi:10.1136/bmj.i6748

Chou, R., Qaseem, A., Snow, V., Casey, D., Cross, J. T., Jr., Shekelle, P., et al. (2007). Diagnosis and treatment of low back pain: a joint clinical practice guideline from the American College of Physicians and the American Pain Society. *Ann. Intern Med.* 147 (7), 478–491. doi:10.7326/0003-4819-147-7-200710020-00006

Conroy, S. M., Courneya, K. S., Brenner, D. R., Shaw, E., O'Reilly, R., Yasui, Y., et al. (2016). Impact of aerobic exercise on levels of IL-4 and IL-10: results from two randomized intervention trials. *Cancer Med.* 5 (9), 2385–2397. doi:10.1002/cam4.836

Cook, M. D., Martin, S. A., Williams, C., Whitlock, K., Wallig, M. A., Pence, B. D., et al. (2013). Forced treadmill exercise training exacerbates inflammation and causes mortality while voluntary wheel training is protective in a mouse model of colitis. *Brain Behav. Immun.* 33, 46–56. doi:10.1016/j.bbi.2013.05.005

Coppola, A., Coppola, L., dalla Mora, L., Limongelli, F. M., Grassia, A., Mastrolorenzo, L., et al. (2005). Vigorous exercise acutely changes platelet and B-lymphocyte CD39 expression. *J. Appl. Physiol.* 98 (4), 1414–1419. doi:10.1152/japplphysiol.00315.2004

Deng, C., and Xia, W. (2018). Effect of Tai Chi Chuan on degeneration of lumbar vertebrae and lumbar discs in middle-aged and aged people: a cross-sectional study based on magnetic resonance images. *J. Int. Med. Res.* 46 (2), 578–585. doi:10.1177/0300060517734115

Djuric, N., Lafeber, G. C. M., and Vleggeert-Lankamp, C. L. A. (2020). The contradictory effect of macrophage-related cytokine expression in lumbar disc herniations: a systematic review. *Eur. Spine J.* 29 (7), 1649–1659. doi:10.1007/s00586-019-06220-w

Djuric, N., Yang, X., Ostelo, R., van Duinen, S. G., Lycklama À Nijeholt, G. J., van der Kallen, B. F. W., et al. (2019). Disc inflammation and Modic changes show an interaction effect on recovery after surgery for lumbar disc herniation. *Eur. Spine J.* 28 (11), 2579–2587. doi:10.1007/s00586-019-06108-9

Driscoll, K. E., Carter, J. M., Hassenbein, D. G., and Howard, B. (1997). Cytokines and particle-induced inflammatory cell recruitment. *Environ. Health Perspect.* 105, 1159–1164. doi:10.1289/ehp.97105s51159

Duzova, H., Karakoc, Y., Emre, M. H., Dogan, Z. Y., and Kilinc, E. (2009). Effects of acute moderate and strenuous exercise bouts on IL-17 production and inflammatory response in trained rats. *J. Sports Sci. Med.* 8 (2), 219–224.

Faelli, E., Bisio, A., Codella, R., Ferrando, V., Perasso, L., Panascì, M., et al. (2020). Acute and chronic catabolic responses to **CrossFit® and resistance training in young males**. *Int. J. Environ. Res. Public Health* 17 (19), 7172. doi:10.3390/ijerph17197172

França, F., Ramos, L., Burke, T., Caffaro, R., and Marques, A. J. A. o.t.R. D. (2013). AB1445-HPR Lumbar stabilization and transcutaneous eletrical nerve stimulation in lumbar disc herniation. *Prelim. study* 71 (Suppl. 3), 757.

Frino, J., McCarthy, R. E., Sparks, C. Y., and McCullough, F. L. J. J. o.P. O. (2006). Trends in adolescent lumbar disk herniation. *Trends Adolesc. lumbar disk herniation* 26 (5), 579–581. doi:10.1097/01.bpo.0000229974.78565.cc

Gulsen, M., Koz, M. J. E. J. o.P. E., and Science, S. (2019). *Effect of proprioceptif neuromuscular facilitation and lumbar stabilization exercises on muscle strength and muscle endurance in patients with lumbar disc hernia.*

Gürşen, C., İnanoğlu, D., Kaya, S., Akbayrak, T., Baltacı, G. J. A. o.g., and obstetrics, H. (2016). Effects of exercise and Kinesio taping on abdominal recovery in women with

cesarean section: a pilot randomized controlled trial. *Arch. Gynecol. Obstet.* 293, 557–565. doi:10.1007/s00404-015-3862-3

Hoffman-Goetz, L., Pervaiz, N., Packer, N., and Guan, J. (2010). Freewheel training decreases pro- and increases anti-inflammatory cytokine expression in mouse intestinal lymphocytes. *Brain Behav. Immun.* 24 (7), 1105–1115. doi:10.1016/j.bbi.2010.05.001

Hoyland, J. A., Freemont, A. J., and Jayson, M. I. (1989). Intervertebral foramen venous obstruction. A cause of periradicular fibrosis? *Spine (Phila Pa 1976)* 14 (6), 558–568. doi:10.1097/00007632-198906000-00002

Iosub, M. E., Ianc, D., Sîrbu, E., Ciobanu, D., and Lazăr, L. J. A. S. (2023). Vojta therapy and conservative physical therapy versus physical therapy only for lumbar disc protrusion: a comparative cohort study from Romania. *Appl. Sci. (Basel).* 13 (4), 2292. doi:10.3390/app13042292

Javaheri, A. A. H., Zadeh, R. K., Kashani, F. O., Mohamadi, J. J. I. J. o.H., and Activity, P. (2011). The effect of combined therapeutic protocol (exercise therapy and massage) on quality of life in male patients suffering from chronic low back pain due to. *Lumbar Disc Herniation* 2 (2), 55–60.

Jeong, D. K., Choi, H. H., Kang, J. I., and Choi, H. (2017). Effect of lumbar stabilization exercise on disc herniation index, sacral angle, and functional improvement in patients with lumbar disc herniation. *J. Phys. Ther. Sci.* 29 (12), 2121–2125. doi:10.1589/jpts.29.2121

Jia, L., Hou, J., Zheng, H., Sun, L., Fan, Y., Wang, X., et al. (2020). Study of the rational dose of propofol in elderly patients under bispectral index monitoring during total intravenous anesthesia: a PRISMA-compliant systematic review. *Med. Baltim.* 99 (5), e19043. doi:10.1097/md.0000000000019043

Kang, J. D., Georgescu, H. I., McIntyre-Larkin, L., Stefanovic-Racic, M., Donaldson, W. F., 3rd, and Evans, C. H. (1996). Herniated lumbar intervertebral discs spontaneously produce matrix metalloproteinases, nitric oxide, interleukin-6, and prostaglandin E2. *Spine (Phila Pa 1976)* 21 (3), 271–277. doi:10.1097/00007632-199602010-00003

Karstoft, K., and Pedersen, B. K. (2016). Exercise and type 2 diabetes: focus on metabolism and inflammation. *Immunol. Cell. Biol.* 94 (2), 146–150. doi:10.1038/icb.2015.101

Katz, J. N. (2006). Lumbar disc disorders and low-back pain: socioeconomic factors and consequences. *J. Bone Jt. Surg. Am.* 88 (Suppl. 2), 21–24. doi:10.2106/jbjs.E.01273

Khanzadeh, R., Mahdavinejad, R., and Borhani, A. (2020). The effect of suspension and conventional core stability exercises on characteristics of intervertebral disc and chronic pain in office staff due to lumbar herniated disc. *Arch. Bone Jt. Surg.* 8 (3), 445–453. doi:10.22038/abjs.2019.40758.2102

Kim, J. H., Kim, Y. E., Bae, S. H., and Kim, K. Y. J. J. o.p.t.s. (2013). The effect of the neurac sling exercise on postural balance adjustment and muscular response patterns in chronic low back pain patients. *J. Phys. Ther. Sci.* 25 (8), 1015–1019. doi:10.1589/jpts.25.1015

Kim, S., Choi, J. Y., Moon, S., Park, D. H., Kwak, H. B., and Kang, J. H. (2019). Roles of myokines in exercise-induced improvement of neuropsychiatric function. *Pflugers Arch.* 471 (3), 491–505. doi:10.1007/s00424-019-02253-8

Kim, Y. S., Park, J., Hsu, J., Cho, K. K., Kim, Y. H., and Shim, J. K. (2010). Effects of training frequency on lumbar extension strength in patients recovering from lumbar dyscectomy. *J. Rehabil. Med.* 42 (9), 839–845. doi:10.2340/16501977-0607

Kraemer, W. J., Ratamess, N. A., and Nindl, B. C. (2017). Recovery responses of testosterone, growth hormone, and IGF-1 after resistance exercise. *J. Appl. Physiol.* 122 (3), 549–558. doi:10.1152/japplphysiol.00599.2016

Kreiner, D. S., Hwang, S. W., Easa, J. E., Resnick, D. K., Baisden, J. L., Bess, S., et al. (2014). An evidence-based clinical guideline for the diagnosis and treatment of lumbar disc herniation with radiculopathy. *Spine J.* 14 (1), 180–191. doi:10.1016/j.spinee.2013.08.003

Lam, O. T., Strenger, D. M., Chan-Fee, M., Pham, P. T., Preuss, R. A., and Robbins, S. M. (2018). Effectiveness of the McKenzie method of mechanical diagnosis and therapy for treating low back pain: literature review with meta-analysis. *J. Orthop. Sports Phys. Ther.* 48 (6), 476–490. doi:10.2519/jospt.2018.7562

Lee, H.-K., Cho, Y.-H., and Lee, J.-C. J. J. o.K. S. o.P. M. (2013). The effect of improve the waist flexibility, the waist muscular strength and the waist balance which grafted in william & McKenzie exercise with Swiss ball. *William Mckenzie Exerc. swiss ball* 8 (4), 479–487. doi:10.13066/kspm.2013.8.4.479

Li, F. J. R. B. d.M. d.E. (2021). The effect of physical exercise on the rehabilitation quality of human waist dysfunction. *Rev. Bras. Med. Esporte* 27, 315–318. doi:10.1590/1517-86922021270320 21_0084

Li, X., Zhan, T., Hu, X., and Qiao, H. J. A. i.C. M. (2022). Study on the effect of rehabilitation exercises on promoting postoperative recovery in patients with lumbar. *Disc Herniation* 12, 5149.

Liu, M., and Gao, S. (2020). Rehabilitation effect of Mulligan technique combined with Swiss ball exercise on the pilots with lumbar disc herniation pain. *J Chin. J. Conval. Med.* 29 (06), 586–590. doi:10.13517/j.cnki.ccm.2020.06.008

Loupasis, G. A., Stamos, K., Katonis, P. G., Sapkas, G., Korres, D. S., and Hartofilakidis, G. (1999). Seven-to 20-year outcome of lumbar discectomy. *Spine (Phila Pa 1976)* 24 (22), 2313–2317. doi:10.1097/00007632-199911150-00005

Lu, W., Kan, S., Shi, H., Li, X., and Hao, Y. J. C. J. o.R. T., (2014). *Effect of proprioceptive training on lumbar disc herniation*, 1162–1164.

Mayer, T. G., Smith, S. S., Keeley, J., and Mooney, V. (1985). Quantification of lumbar function. Part 2: sagittal plane trunk strength in chronic low-back pain patients. *Spine (Phila Pa* 10 (8), 765–772. doi:10.1097/00007632-198510000-00012

McGill, S. M. J. P. t. (1998). *Low back exercises: evidence for improving exercise regimens*.

Meng, Z., Zheng, J., Fu, K., Kang, Y., and Wang, L. J. J. o.H. E. (2022). *Curative effect of foraminal endoscopic surgery and efficacy of the wearable lumbar spine protection equipment in the treatment of lumbar disc herniation*.

Merritt, L. G., and Merritt, C. M. (2007). The gym ball as a chair for the back pain patient: a two case report. *J. Can. Chiropr. Assoc.* 51 (1), 50–55.

Mitchell, J. B., Paquet, A. J., Pizza, F. X., Starling, R. D., Holtz, R. W., and Grandjean, P. W. (1996). The effect of moderate aerobic training on lymphocyte proliferation. *Int. J. Sports Med.* 17 (5), 384–389. doi:10.1055/s-2007-972865

Miyamoto, H., Saura, R., Harada, T., Doita, M., and Mizuno, K. (2000). The role of cyclooxygenase-2 and inflammatory cytokines in pain induction of herniated lumbar intervertebral disc. *Kobe J. Med. Sci.* 46 (1-2), 13–28.

Naylor, A., Happey, F., Turner, R. L., Shentall, R. D., West, D. C., and Richardson, C. (1975). Enzymic and immunological activity in the intervertebral disc. *Orthop. Clin. North Am.* 6 (1), 51–58. doi:10.1016/s0030-5898(20)31199-8

Nieman, D. C., and Pedersen, B. K. (1999). Exercise and immune function. Recent developments. *Sports Med.* 27 (2), 73–80. doi:10.2165/00007256-199927020-00001

Norling, A. M., Gerstenecker, A. T., Buford, T. W., Khan, B., Oparil, S., and Lazar, R. M. (2020). The role of exercise in the reversal of IGF-1 deficiencies in microvascular rarefaction and hypertension. *Geroscience* 42 (1), 141–158. doi:10.1007/s11357-019-00139-2

Parke, W. W., and Watanabe, R. (1985). The intrinsic vasculature of the lumbosacral spinal nerve roots. *Spine (Phila Pa 1976)* 10 (6), 508–515. doi:10.1097/00007632-198507000-00004

Peake, J. M., Suzuki, K., Hordern, M., Wilson, G., Nosaka, K., and Coombes, J. S. (2005). Plasma cytokine changes in relation to exercise intensity and muscle damage. *Eur. J. Appl. Physiol.* 95 (5-6), 514–521. doi:10.1007/s00421-005-0035-2

Pelosi, L., Giacinti, C., Nardis, C., Borsellino, G., Rizzuto, E., Nicoletti, C., et al. (2007). Local expression of IGF-1 accelerates muscle regeneration by rapidly modulating inflammatory cytokines and chemokines. *Faseb J.* 21 (7), 1393–1402. doi:10.1096/fj.06-7690com

Pichon, A. P., Connes, P., and Robach, P. (2016). Effects of acute and chronic hematocrit modulations on blood viscosity in endurance athletes. *Clin. Hemorheol. Microcirc.* 64 (2), 115–123. doi:10.3233/ch-162050

Rahimi, A. J. J. o.P. A., and Hormones, H. (2019). Eight weeks resistance training reduces interlukin-17 in women with multiple sclerosis. 2(4), 27–38.

Rojas Vega, S., Knicker, A., Hollmann, W., Bloch, W., and Strüder, H. K. (2010). Effect of resistance exercise on serum levels of growth factors in humans. *Horm. Metab. Res.* 42 (13), 982–986. doi:10.1055/s-0030-1267950

Rydevik, B., Brown, M. D., and Lundborg, G. (1984). Pathoanatomy and pathophysiology of nerve root compression. *Spine (Phila Pa 1976)* 9 (1), 7–15. doi:10.1097/00007632-198401000-00004

Saal, J. A. (1996). Natural history and nonoperative treatment of lumbar disc herniation. *Spine (Phila Pa 1976)* 21 (24 Suppl. l), 2S-9S–9s. doi:10.1097/00007632-199612151-00002

Selim, M. N., Mokhtar, M. M., Mohamed, M. H., Abonour, A. A., Abdelmutilibe, S. M., and Essa, M. M. J. S. T.-R. E. d.C. d.D. (2022). *Comparison between efficacy of spinal mobilization with leg movement versus McKenzie technique in patients with lumbar disc herniation*, 23.

Shamji, M. F., Setton, L. A., Jarvis, W., So, S., Chen, J., Jing, L., et al. (2010). Proinflammatory cytokine expression profile in degenerated and herniated human intervertebral disc tissues. *Arthritis Rheum.* 62 (7), 1974–1982. doi:10.1002/art.27444

Shen, Z., Tu, Q., and Liu, Z. (2009). The influence of Swiss ball exercises on pain symptom and muscle strength in patients with protrusion of lumber intervertebral disc. *J Chin. J. Rehabilitation Med.* 24 (12), 1103–1105.

Sipaviciene, S., and Kliziene, I. J. C. b. (2020). Effect of different exercise programs on non-specific chronic low back pain and disability in people who perform sedentary work. *Clin. Biomech.* 73, 17–27. doi:10.1016/j.clinbiomech.2019.12.028

Stevens, V. K., Coorevits, P. L., Bouche, K. G., Mahieu, N. N., Vanderstraeten, G. G., and Danneels, L. A. (2007). The influence of specific training on trunk muscle recruitment patterns in healthy subjects during stabilization exercises. *Man. Ther.* 12 (3), 271–279. doi:10.1016/j.math.2006.07.009

Tan, J. H., Li, Z. P., Liu, L. L., Liu, H., and Xue, J. B. J. C. B. I. (2022). IL-17 in intervertebral disc degeneration: mechanistic insights and therapeutic implications. *Cell. Biol. Int.* 46 (4), 535–547. doi:10.1002/cbin.11767

Taşpınar, G., Angın, E., and Oksüz, S. J. J. o.C. E. R. (2023). The effects of Pilates on pain, functionality, quality of life, flexibility and endurance in lumbar disc herniation. *J. Comp. Eff. Res.* 12 (1), e220144. doi:10.2217/cer-2022-0144

Uçeyler, N., Tscharke, A., and Sommer, C. (2007). Early cytokine expression in mouse sciatic nerve after chronic constriction nerve injury depends on calpain. *Brain Behav. Immun.* 21 (5), 553–560. doi:10.1016/j.bbi.2006.10.003

Wang, S. Q., Yang, X. Y., Cui, S. X., Gao, Z. H., and Qu, X. J. (2019). Heterozygous knockout insulin-like growth factor-1 receptor (IGF-1R) regulates mitochondrial functions and prevents colitis and colorectal cancer. *Free Radic. Biol. Med.* 134, 87–98. doi:10.1016/j.freeradbiomed.2018.12.035

Weyand, C. M., and Goronzy, J. J. (2021). The immunology of rheumatoid arthritis. *Nat. Immunol.* 22 (1), 10–18. doi:10.1038/s41590-020-00816-x

Wipfli, B., Landers, D., Nagoshi, C., and Ringenbach, S. (2011). An examination of serotonin and psychological variables in the relationship between exercise and mental health. *Scand. J. Med. Sci. Sports* 21 (3), 474–481. doi:10.1111/j.1600-0838.2009.01049.x

Xu, J., Ding, X., Wu, J., Zhou, X., Jin, K., Yan, M., et al. (2020). A randomized controlled study for the treatment of middle-aged and old-aged lumbar disc herniation by Shis spine balance manipulation combined with bone and muscle guidance. *Med. Baltim.* 99 (51), e23812. doi:10.1097/md.0000000000023812

Yildirim, P., and Gultekin, A. J. S. (2022). The effect of a stretch and strength-based yoga exercise program on patients with neuropathic pain due to lumbar disc herniation. *Lumbar Disc Herniation* 47 (10), 711–719. doi:10.1097/BRS.0000000000004316

Yorimitsu, E., Chiba, K., Toyama, Y., and Hirabayashi, K. (2001). Long-term outcomes of standard discectomy for lumbar disc herniation: a follow-up study of more than 10 years. *Spine (Phila Pa* 26 (6), 652–657. doi:10.1097/00007632-200103150-00019

Żebrowska, A., Hall, B., Maszczyk, A., Banaś, R., and Urban, J. (2018). Brain-derived neurotrophic factor, insulin like growth factor-1 and inflammatory cytokine responses to continuous and intermittent exercise in patients with type 1 diabetes. *Diabetes Res. Clin. Pract.* 144, 126–136. doi:10.1016/j.diabres.2018.08.018

Zheng, K., Wen, Z., and Li, D. J. J. o.h.e. (2021). *The clinical diagnostic value of lumbar intervertebral disc herniation based on MRI images*.

Zhou, X., Kong, L., Ren, J., Song, P., Wu, Z., He, T., et al. (2022). Effect of traditional Chinese exercise combined with massage on pain and disability in patients with lumbar disc herniation: a multi-center, randomized, controlled, assessor-blinded clinical trial. *Front. Neurol.* 13, 952346. doi:10.3389/fneur.2022.952346

Zou, L., Zhang, Y., Liu, Y., Tian, X., Xiao, T., Liu, X., et al. (2019). The effects of tai chi chuan versus core stability training on lower-limb neuromuscular function in aging individuals with non-specific chronic lower back pain. *Med. Kaunas.* 55 (3), 60. doi:10.3390/medicina55030060

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 22 January 2024
DOI 10.3389/fphys.2024.1268380

OPEN ACCESS

EDITED BY
Tania Gamberi,
University of Florence, Italy

REVIEWED BY
Susan Tsivitse Arthur,
University of North Carolina at Charlotte, United States
Vanessa Azevedo Voltarelli,
Hospital Sirio Libanes, Brazil
Teppei Fujikawa,
University of Texas Southwestern Medical Center, United States

*CORRESPONDENCE
Wilian A. Silveira,
wilian.silveira@uftm.edu.br

†These authors have contributed equally to this work

RECEIVED 27 July 2023
ACCEPTED 08 January 2024
PUBLISHED 22 January 2024

CITATION
Abdalla-Silva RL, Zanetti GO, Lautherbach N, Schavinski AZ, Heck LC, Gonçalves DAP, Kettelhut IC, Navegantes LCC and Silveira WA (2024), β_2-Adrenoceptors activation regulates muscle trophic-related genes following acute resistance exercise in mice.
Front. Physiol. 15:1268380.
doi: 10.3389/fphys.2024.1268380

β_2-Adrenoceptors activation regulates muscle trophic-related genes following acute resistance exercise in mice

Ronaldo L. Abdalla-Silva[1†], Gustavo O. Zanetti[2], Natalia Lautherbach[3,4], Aline Zanatta Schavinski[3], Lilian C. Heck[3], Dawit A. P. Gonçalves[2,5], Isis C. Kettelhut[4], Luiz C. C. Navegantes[3] and Wilian A. Silveira[1*†]

[1]Department of Biochemistry, Pharmacology and Physiology, Institute of Biological and Natural Sciences, Federal University of Triângulo Mineiro, Uberaba, Minas Gerais, Brazil, [2]Exercise Physiology Laboratory, School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [3]Department of Physiology, Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil, [4]Department of Biochemistry/Immunology, Ribeirão Preto Medical School, University of São Paulo, São Paulo, Brazil, [5]Sports Training Center, School of Physical Education, Physiotherapy and Occupational Therapy, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Resistance exercise (RE) training and pharmacological stimulation of β_2-Adrenoceptors (β_2-ARs) alone can promote muscle hypertrophy and prevent muscle atrophy. Although the activation of the sympathetic nervous system (SNS) is a well-established response during RE, the physiological contribution of the endogenous catecholamines and β_2-ARs to the RE-induced changes on skeletal muscle protein metabolism remains unclear. This study investigated the effects of the β_2-ARs blockade on the acute molecular responses induced by a single bout of RE in rodent skeletal muscles. Male C57BL6/J mice were subjected to a single bout of progressive RE (until exhaustion) on a vertical ladder under β_2-AR blockade with ICI 118,551 (ICI; 10 mg kg^{-1}, i. p.), or vehicle (sterile saline; 0.9%, i. p.), and the gene expression was analyzed in *gastrocnemius* (GAS) muscles by qPCR. We demonstrated that a single bout of RE acutely increased the circulating levels of stress-associated hormones norepinephrine (NE) and corticosterone (CORT), as well as the muscle phosphorylation levels of AMPK, p38 MAPK and CREB, immediately after the session. The acute increase in the phosphorylation levels of CREB was followed by the upregulation of CREB-target genes *Sik1*, *Ppargc1a* and *Nr4a3* (a central regulator of the acute RE response), 3 h after the RE session. Conversely, β_2-AR blockade reduced significantly the *Sik1* and *Nr4a3* mRNA levels in muscles of exercised mice. Furthermore, a single bout of RE stimulated the mRNA levels of the atrophic genes *Map1lc3b* and *Gabarapl1* (autophagy-related genes) and *Mstn* (a well-known negative regulator of muscle growth). Unexpectedly, the gene expression of *Igf-1* or *Il-6* were not affected by RE, while the atrophic genes *Murf1/Trim63* and *Atrogin-1/Mafbx32* (ubiquitin-ligases) were increased only in muscles of exercised mice under β_2-AR blockade. Interestingly, performing a single bout of RE under β_2-AR blockade increased the mRNA levels of *Mstn* in muscles of exercised mice. These data suggest that β_2-ARs stimulation during acute RE stimulates the hypertrophic gene *Nr4a3* and prevents the overexpression of atrophic genes such as *Mstn*, *Murf1/Trim63*,

and *Atrogin-1/Mafbx32* in the first hours of postexercise recovery, indicating that he SNS may be physiologically important to muscle adaptations in response to resistance training.

KEYWORDS

resistance exercise, β2-adrenoceptor, skeletal muscle, myostatin, NR4A3

1 Introduction

Skeletal muscle is the most abundant and plastic tissue in the human body, comprising approximately 40%–50% of total body mass (Sartori et al., 2021). This tissue is fundamental for locomotion, breathing, thermogenesis, energy expenditure, and glycemic control (Pedersen, 2013; Schnyder and Handschin, 2015). Due to these important functions, muscle wasting and weakness have been associated with reduced quality of life and higher mortality risks of all causes, cancer, chronic obstructive pulmonary disease (COPD) and aging (Mathur et al., 2014; Santana et al., 2019; Zhou et al., 2023). Despite these alarming findings, there is no effective pharmacological treatment for preventing muscle atrophy in such conditions.

Resistance exercise (RE) training, on the other hand, may increase muscle mass (hypertrophy) and strength (Hornberger and Farrar, 2004; Ruas et al., 2012) and prevent muscle wasting in catabolic situations, such as cancer, glucocorticoids treatment, and sarcopenia (Kim et al., 2016; Padilha et al., 2021; Testa et al., 2022; Macedo et al., 2023). These long-term adaptations to RE training involve acute and transient changes in mRNA expression of various genes in response to each single bout of exercise, including the muscle-derived myokines insulin-like growth factor 1 (IGF-1), myostatin (MSTN) and interleukin 6 (IL-6), among others (Piccirillo, 2019). Such changes in mRNA expression occur between 3h and 12 h after an exercise bout and result in a gradual modification in protein content and activity (Egan and Zierath, 2013). Ultimately, the transcriptional and post-translational regulation induced by RE leads to muscle growth, when the rate of protein synthesis exceeds protein degradation over time (Phillips et al., 1997), while reduced rates of protein degradation seem to be necessary for attenuating muscle atrophy (Graham et al., 2021). Although the health benefits of regular RE training on skeletal muscle physiology are well established, the acute molecular mechanism and signaling pathways controlling protein metabolism after a single bout of RE remains elusive.

Most physiological systems of the body (i.e., nervous, cardiovascular, respiratory, endocrine and musculoskeletal) are stimulated during RE (Athanasiou et al., 2023). For example, the RE acutely increases the secretion of catabolic hormones such as glucocorticoids (e.g., cortisol in humans; corticosterone in rodents) and reduces the anabolic hormone insulin (INS) (Kraemer and Ratamess, 2005; Athanasiou et al., 2023), extracellular alterations associated with decreased protein kinase B (PKB)/Akt and mitogen-activated protein kinases (MAPKs) ERK1/2 phosphorylation/activation in skeletal muscle (Osman et al., 2000; Yang et al., 2008; Hu et al., 2009). On one hand, the dephosphorylation of these intracellular mediators of the INS can activate the Forkhead box class O (*i.e.,* FoxO), leading to transcription of several components of the ubiquitin-proteasome (UPS; *Atrogin-1/Mafbx32* and *MuRF1/Trim63*) and autophagic-lysosomal (ALS; *Map1lc3b* and *Gabarapl1*) systems, which degrade most cellular proteins and organelles in skeletal muscle (Yang et al., 2008; Milan et al., 2015). Accordingly, the rate of protein degradation increases immediately after the cessation of an acute RE session, probably to prevent the accumulation of damaged proteins and organelles (Phillips et al., 1997; Grumati et al., 2011; Aweida and Cohen, 2021). On the other hand, decreased Akt and ERK1/2 phosphorylation and their downstream targets GSK-3 and mTOR activities are associated with reduced protein synthesis (Rommel et al., 2001; Figueiredo and Markworth, 2015; Miyazaki and Takemasa, 2017). Despite that, enhanced myofibrillar and mitochondrial protein synthesis appear to be a critical step in the recovery of muscle homeostasis in the hours after acute RE (Wilkinson et al., 2008; Egan and Zierath, 2013). Even though the molecular mechanisms involved in the increase of the protein synthesis are well characterized, the role of the proteolytic systems after a single bout of RE and during adaptive muscle hypertrophy is not completely understood.

As a physiological stress, RE also stimulates the activity of the sympathetic nervous system (SNS), a branch of the autonomic nervous system (ANS), to meet the metabolic demand of active skeletal muscles during exercise (Athanasiou et al., 2023). Indeed, RE stimulates the sympathoadrenal axis to increase the plasma catecholamines levels (epinephrine [EPI] and norepinephrine [NE]) as a function of exercise intensity (Kraemer and Ratamess, 2005; Athanasiou et al., 2023), which return to the basal levels between 5 and 15 min after the end of exercise (Goto et al., 2008; Fatouros et al., 2010). Moreover, it has been shown that muscle sympathetic nerve activity (MSNA) also increases in proportion to the rise in intensity (Katayama and Saito, 2019). Based on that, the adrenergic actions of SNS can be mediated by a hormonal (catecholamines released from adrenal medulla) and/or a neural mechanism (NE secreted from sympathetic nerve ends) in many tissues, including skeletal muscle (Graça et al., 2013; Khan et al., 2016; Rodrigues et al., 2019).

In skeletal muscle, there is a significant proportion of GTP-binding protein stimulatory (Gαs)-coupled ß-adrenoceptors (β-ARs), predominantly of β_2-subtype (β_2-AR; ~90%), that can be activated by endogenous catecholamines via circulation and/or direct muscle noradrenergic innervation (Elfellah et al., 1989; Lynch and Ryall, 2008; Khan et al., 2016). Upon binding of catecholamines, Gαs-coupled β_2-ARs activate adenylyl cyclase (AC), leading to the increase in intracellular second messenger cyclic adenosine monophosphate (cAMP), and subsequent activation of cAMP-dependent protein kinase (PKA) (Lynch and Ryall, 2008). Once activated, the free catalytic subunits of PKA phosphorylate several substrates, including regulatory enzymes (*i.e.,* glycogen synthetase and hormone-sensitive lipase), the ryanodine receptor (RyR), and the cAMP response element-binding protein

(CREB) transcription factor, resulting in enhanced catabolism of energy substrates, muscle contractility, and gene expression, respectively (Altarejos and Montminy, 2011; Berdeaux and Stewart, 2012; Cairns and Borrani, 2015). In contrast to these acute effects of catecholamines, chronic treatment with β_2-AR agonists promotes muscle growth and can be used for attenuating muscle wasting (Kim and Sainz, 1992; Mersmann, 1998; Yimlamai et al., 2005; Harcourt et al., 2007; Ryall et al., 2007). The molecular mechanisms by which β_2-AR agonists induce these anabolic and anti-catabolic effects on skeletal muscle are not completely understood but may involve canonical (i.e., cAMP/PKA) and/or non-canonic signaling pathways (Lynch and Ryall, 2008; Gonçalves et al., 2012; Silveira et al., 2020). Indeed, it has been postulated that β_2-AR agonists stimulate muscle hypertrophy and prevent muscle atrophy by activating Akt/mTOR and Akt/FoxO pathways, respectively, via the subunits βγ of GTP-binding inhibitory protein-coupled β_2-AR (Giα-Gβγ) (Xiao, 2001; Kline et al., 2007; Lynch and Ryall, 2008; Koopman et al., 2010). Moreover, β_2AR-induced skeletal muscle growth and strength might also be mediated by *ß*-arrestin 1, a multifunctional adaptor protein that function as a signal transducer required for the activation of other signaling molecules in muscle cells, such as p38 MAPK, ERK1/2, CREB and AMPK (Kim et al., 2018). On the other hand, the canonical signaling pathway cAMP/PKA seems to be important to maintain muscle mass under basal conditions by restraining FoxO transcriptional activity and the expression of multiple components of the UPS and ALS (Silveira et al., 2020). Moreover, these β_2-AR agonists also induce a shift in muscle composition from slow to fast fiber type (Kim et al., 2018; Gonçalves et al., 2019). Thus, although RE training and pharmacological stimulation of β_2-ARs alone promote muscle remodeling and adaptation, the physiological role of endogenous catecholamines and β_2-ARs in mediating muscle anabolism to RE remains unknown. Therefore, the current study was undertaken to investigate the effects of the β_2-ARs blockade on the acute molecular changes induced by a single bout of RE in rodent skeletal muscles. By using a pharmacological approach, we demonstrate that the β_2-AR activation during RE is required to induce *Nr4a3* and to prevent the overexpression of atrophic genes such as *Mstn*, *Murf1/Trim63*, and *Atrogin-1/Mafbx32*.

2 Material and methods

2.1 Animals and treatments

C57Bl6/J mice (8-week-old male mice, ~20–23 g) were housed in a room with a 12–12 h light–dark cycle (starting at 6:00 a.m.) and given free access to water and a normal lab chow diet (Nuvilab-CR1; Nuvital, Curitiba, PR, Brazil) until the start of the experiments. The sample size used for each experiment is indicated in the figure legends. The animals were randomly divided into two or three groups as follows: control (CON) and resistance exercise (RE) (experiment one); or CON, RE and RE under β_2-AR blockade (ICI + RE) (experiment two). Mice from ICI + RE group were treated intraperitoneally (i.p.) with 10 mg kg^{-1} of the selective β_2-AR antagonist (ICI 118,551; Sigma-Aldrich) 30 min before the RE bout (Azevedo Voltarelli et al., 2021), whereas mice from CON and RE groups were injected with vehicle (0.9% saline). Immediately after (0 h) and 3 hours (3 h) after exercise cessation, the animals were anesthetized with isoflurane by using the open-drop technique (Risling et al., 2012) and euthanized by decapitation with the aid of a rodent guillotine for collecting blood and muscle samples (experiment one) or by cervical dislocation when collecting muscle samples only (experiment two). For this, when the animal was fully anesthetized, the thumb and index finger were positioned on either side of the neck at the base of the skull. With the other hand, the base of the tail was quickly pulled, causing separation of the cervical vertebrae from the skull. After euthanasia, muscles were rapidly harvested, weighed, frozen in liquid nitrogen, and stored at −80°C until further analyses. All experiments were performed accordingly to the ethical principles for animal research adopted by the Brazilian College of Animal Experimentation and were approved by the Ribeirão Preto Medical School from the University of São Paulo - Commission of Ethics in Animal Research (no. 207/2014).

2.2 Experimental design

As above mentioned, the present study was divided into two experiments. Experiment one was performed to test whether the acute RE protocol would be able to increase sympathetic activity. For that, the animals were randomly divided into two groups: 1) CON and 2) RE groups. After the familiarization period with the ladder apparatus for RE and the maximum voluntary carrying capacity (MVCC) test, mice from the RE group were subjected to a single bout of RE (see *Resistance Exercise Protocol* section). Immediately after (0 h) the exercise session, the animals were anesthetized with isoflurane and euthanized by decapitation for collecting blood and muscle samples in order to analyze plasma catecholamines by HPLC and muscle proteins content, and phosphorylation by immunoblotting. Experiment two was performed to determine the contribution of β_2-ARs to skeletal muscular transcriptional response following a single bout of RE. For that, the animals were randomly divided into three groups: 1) CON, 2) RE, and 3) ICI + RE. After the familiarization period with the ladder apparatus for RE and MVCC test, mice from RE and ICI + RE groups were submitted to the RE protocol. Three hours (3 h) after the exercise session, the animals were anesthetized with isoflurane and euthanized by cervical dislocation, and muscles were rapidly harvested and stored at −80°C until qPCR and Western blotting analysis. The 3 h period was chosen because rodents subjected to a single bout of exercise showed transient changes in mRNA levels in the first hours of recovery, typically between 3h and 12 h after exercise cessation (Egan and Zierath, 2013; Egan and Sharples, 2023). Mice from the CON group remained confined in their cages during both experimental protocols, except when all animals were submitted to the familiarization period with the ladder apparatus for RE and MVCC test.

2.3 Resistance exercise protocol

The resistance exercise (RE) protocol was based on previous reports (Frajacomo et al., 2015; Padilha et al., 2019) and consisted of

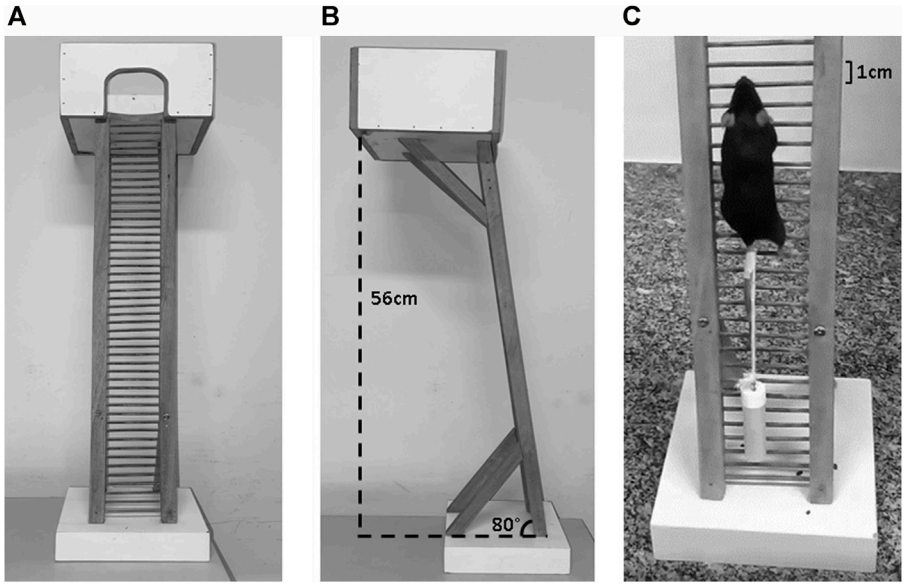


FIGURE 1
(A) The apparatus for resistance exercise (RE) in mice. **(B)** At the top of the ladder is a housing chamber where the mice are allowed to rest between climbs (60 s). **(C)** A mouse is shown climbing a 56 cm, 80° incline ladder with the weight attached to the tail.

ladder climbing (80° incline; 1.0 cm space between steps, and 56 cm height) using progressive overload (see Figures 1A–C). Initially, all mice were familiarized to voluntary climbing the ladder over five consecutive days. For this, mice were positioned on the base of the ladder and encouraged to climb by pushing them to initiate movements. These stimuli were applied until each animal was able to complete an entire climb (Padilha et al., 2017). Forty-8 h after the last familiarization session, the animals were submitted to MVCC test, which consisted of carrying loads equivalent to 50%, 75%, 90%, and 100% of body mass attached to the tail of each mouse. When the animal reached the covered chamber (Figure 1A), an interval of 60 s was given between each climbing bout. After climbing the ladder with 100% of its body mass, a 3 g load was added for the next climbs until a load that incapacitated the animal to fully climb the ladder length. The maximum load (MAX) achieved in the MVCC test was used as a parameter to determine the carrying load during the acute RE session. Finally, 48 h after MVCC test, only the animals from RE and ICI + RE groups were submitted to MAX-based protocol of acute RE (50% of MAX, 75%, 90%, 100%, 100% plus 3 g until failure). Failure was defined as the incapacity to carry the weight even after two successive attempts. All animals remained food deprived from the start of the experiments until euthanasia (about 3.5 h).

2.4 Catecholamines and corticosterone levels

Both plasma catecholamines, epinephrine (EPI) and norepinephrine (NE), and the tissue NE from *gastrocnemius* (GAS) and *tibialis anterior* (TA) muscles were assayed as previously described (Garofalo et al., 1996) using HPLC (LC-7A, Shimadzu Instruments) with a 5-μm Spherisorb ODS-2 reversed-phase column (Sigma-Aldrich). Serum corticosterone levels were measured by specific radioimmunoassay as previously described (Durlo et al., 2004).

TABLE 1 Antibodies for Western blot.

Protein	Dilution for WB	Manufacturer
phospho-Ser^{133} CREB	1:2000	Cell Signaling
phospho-Ser/Thr PKA	1:1000	Cell Signaling
phospho-Thr^{172} AMPK	1:1000	Cell Signaling
phospho-Ser^{256} FoxO1	1:1000	Cell Signaling
phospho-Thr^{24} FoxO1	1:1000	Cell Signaling
phospho-Thr^{32} FoxO3	1:1000	Cell Signaling
phospho-Thr^{202} ERK1	1:2000	Cell Signaling
phospho-Tyr^{204} ERK2	1:2000	Cell Signaling
phospho-Thr^{180}/Tyr^{182} p38-MAPK	1:1000	Cell Signaling
phospho-Ser^{473} AKT	1:1000	Cell Signaling
phospho-Ser$^{235/236}$ S6	1:1000	Cell Signaling
phospho-Ser$^{240/244}$ S6	1:1000	Cell Signaling
phospho-Ser^{21} GSK3α	1:1000	Cell Signaling
phospho-Ser^{9} GSK3β	1:1000	Cell Signaling
phospho-Thr^{286} CaMKII	1:1000	Cell Signaling
Atrogin-1	1:1000	Santa Cruz
α- Tubulin	1:2000	Santa Cruz
β-actin	1:2000	Santa Cruz

TABLE 2 Oligonucleotide primers used for qPCR analysis.

Gene	Forward primer (5′-3′)	Reverse primer (3′-5′)
Ppargc1α	AATCCAGCGGTCTTAGCACT	TTTCTGTGGGTTTGGTGTGA
Sik1	TCCACCACCAAATCTCACCG	GTTTCGGCGCTGCCTCTTC
Map1lc3b	CGTCCTGGACAAGACCAAGT	TCCGTCCTTCGCTTCATAGG
Gabarapl1	CATCGTGGAGAAGGCTCCTA	ATACAGCTGGCCCATGGTAG
Ctsl	CATCGTGGAGAAGGCTCCTA	ATACAGCTGGCCCATGGTAG
Bnip3	TTCCACTAGCACCTTCTGATGA	GAACACCGCATTTACAGAACAA
Nr4a1	AGCTTGGGTGTTGATGTTCC	AATGCGATTCTGCAGCTCTT
Nr4a3	TGCAGAGCCTGAACCTTGAT	TTAACCCATGTCGCTCTGTG
Mstn	TTGCAAAATTGGCTCAAACAGC	AAGGGATTCAGCCCATCTTCTC
Igf-1	CTCAGACAGGCATTGTGGATGAGT	GGTCTTGTTTCCTGCACTTCCTCT
Il-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Murf1	TGTGCAAGGAACACGAAG	TGAGAGATGATCGTCTGC
Atrogin-1	GCAGAGAGTCGGCAAGTC	CAGGTCGGTGATCGTGAG
Adr2	GAGCACAAAGCCCTCAAGAC	TGGAAGGCAATCCTGAAATC
Myh1	ACCTTGTGGACAAACTGCAA	AGCTTGTTGACCTGGGACTC
Myh2	TTGGTGGATAAACTCCAGGC	CAGCTTGTTGACCTGGGACT
Myh4	ACAGACTAAAGTGAAAGCCTACAA	CACATTTTGTGATTTCTCCTGTCAC
Myh7	AGCAGGAGCTGATTGAGACC	TGTGATAGCCTTCTTGGCCT
Rpl39	TCCTGGCAAAGAAACAAAAGC	TAGACCCAGCTTCGTTCTCCT

2.5 Western blotting analysis

GAS and TA muscles were homogenized in RIPA buffer containing 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM sodium orthovanadate, 5 μg mL^{-1} of aprotinin, 1 mg mL^{-1} of leupeptin, and 1 mM phenylmethyl-sulfonyl fluoride (PMSF). Lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted using antibodies listed in Table 1. Primary antibodies were detected using peroxidase-conjugated secondary antibodies and visualized using enhanced chemiluminescence (ECL) reagents on ChemiDoc XRS + System (Bio-Rad). Band intensities were quantified using the software ImageJ/Fiji (version 1.52 d, National Institutes of Health, United States).

2.6 Real-time qPCR

The analysis of qPCR was performed at the Laboratory of Metabolism Control from Ribeirão Preto Medical School (University of São Paulo). After the exercise protocol described above, GAS muscles were immediately frozen in liquid nitrogen and stored at −80°C. Total RNA was extracted from muscle using TRIzol (50 mg of muscle was added to 0.5 mL of TRIzol, Invitrogen®). Samples were homogenized in tubes using a TissueLyser II (Qiagen®) with 5 mm stainless steel beads for 2 × 1 min cycles at 30 Hz, resting on ice in between. Homogenates were cleared by centrifugation at 10,000 x g for 5 min at 4°C. RNA extraction was performed according to TRIzol manufacturer's instructions (Invitrogen®). RNA was eluted in 50 μL of RNase-free water and stored at − 80°C. RNA samples were treated with DNase I, RNase-free (Thermo Fisher Scientific®), to remove genomic DNA contamination. RNA samples were quantitated using NanoDrop One spectrophotometer (Thermo Fisher Scientific®), following the manufacturer's instructions. The same device was used to assess the purity of RNA by measuring 260/280 and 260/230 ratios of absorbance values. Samples presenting a 260/280 ratio of ~2 and 260/230 ratio of 2–2.2 were accepted as "pure" for RNA. According to the manufactures' protocols, 1 μg of RNA was reverse transcribed into cDNA using 0.5 μL of SuperScript IV First-Strand Synthesis System (Invitrogen®). cDNA was diluted 25-fold with nuclease-free water. For qPCR, the total volume per reaction was 10 μL containing 5 μL of cDNA (2 ng/μL), 4.8 μL of PowerUp SYBR Green Master Mix (Thermo Fisher®), and 0.2 μL of primers (forward and reverse mixture; 50 μmol/L stock; Table 2). qPCR run on Applied Biosystems™ 7,500 Real-Time PCR System, using the recommended cycling conditions as follows: a pre-incubation of 2 min at 50°C and 10 min at 95°C, followed by a two-step amplification program of 40 cycles set at 95 °C for 15 s (denaturation) and 60 °C for 1 min (annealing + extension) and, finally, a dissociation stage set at 95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s. The last stage was performed to evaluate the quality of qPCR reactions regarding of nonspecific amplification and primer-dimer formation in a dissociation curve for each gene. The amplification specificity for each primer was confirmed by

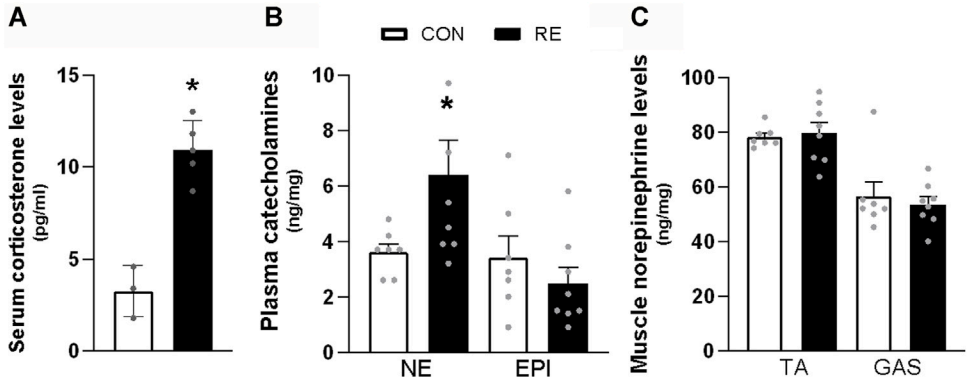


FIGURE 2
Acute effect of a single bout of resistance exercise (RE) on the activation of sympathetic-adrenomedullary and the hypothalamus-pituitary-adrenal (HPA) axis of mice. **(A)** Serum corticosterone, **(B)** plasma catecholamines, and **(C)** content of norepinephrine in *tibialis anterior* (TA) and *gastrocnemius* (GAS) muscles of mice, immediately after (0 h) the RE session. Data are presented as mean ± SEM of 7-8 mice. (*$p \leq 0.05$ vs CON group, Student's t-test). NE, norepinephrine; EPI, epinephrine.

observing the single melt curve peak after the completion of qPCR. Primer sequences were designed utilizing Primer3Plus (https://www.primer3plus.com/) in conjunction with OligoAnalyzer 3.1 (https://eu.idtdna.com/site) and cross-referenced using the Basic Local Alignment Search Tool program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A six-point relative standard curve was prepared for each gene by using five-fold serial dilutions of pooled cDNA samples in duplicate. No threshold cycle quantification value for the no template control was detected. The relative expression levels of target genes were calculated using the $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak, 2008). Data from the target genes were normalized by the expression of *Rpl39* as a reference gene.

2.7 Statistics

The distribution and variance homogeneity were tested using Shapiro-Wilk test. The data are expressed as means ± standard error mean (SEM). According to each experimental design, non-paired Student's t-test or one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* were used in normally distributed variables or those that showed a normal distribution after log transformation. Kruskal–Wallis test followed by Dunn's *post hoc* test was used for non-parametric data. Data were analyzed using JASP 0.16.2 software (GNU Affero GPL v3 license, Department of Psychological Methods, University of Amsterdam) and GraphPad Prism version 7.0 (Graph Pad Softwares, San Diego, CA, United States). The significance level adopted was 5% ($p \leq 0.05$).

3 Results

3.1 A single bout of RE acutely increases the circulating levels of norepinephrine and corticosterone

Since it has been demonstrated that RE may stimulate a stress response by activating both the sympathetic-adrenomedullary and the hypothalamus-pituitary-adrenal (HPA) axis (Kraemer and Ratamess, 2005; Athanasiou et al., 2023), we first investigated the acute effect of the ladder climbing-based RE protocol on circulating catecholamines and corticosterone levels, immediately after (0 h) RE cessation. As expected, a single bout of RE acutely increased the serum corticosterone (~3-fold) and plasma NE (~2-fold) levels (Figures 2A, B). On the other hand, plasma EPI levels were not altered (Figure 2A). Moreover, because muscle sympathetic nerve activity (MSNA) has also been reported to increase during exercise (Katayama and Saito, 2019), we evaluated the NE levels in GAS and TA muscles of mice immediately after (0 h) the RE cessation. As shown in Figure 2C, the NE levels in both muscles were unchanged following RE.

3.2 A single bout of RE acutely stimulates intracellular pathways involved in energy metabolism and adrenergic signaling in skeletal muscle

Because RE stimulated systemic SNS activity, we examined whether the canonical ß-AR signaling pathway (*i.e.,* PKA/CREB) was also activated in the muscles of exercised mice immediately after (0 h) the RE cessation. Although TA muscles were unaffected, a single bout of RE acutely increased the phosphorylation levels of CREB (~30%) in GAS muscles, without altering the phosphorylation levels of other PKA substrates at Ser/Thr (Figures 3A–C). These findings suggest that the activity of transcription factor CREB was increased, but other upstream kinases than PKA may have also been responsible for such an effect. In addition to PKA, the protein kinases AMPK, CAMKII, and p38 MAPK (via mitogen-and stress-activated kinase 1; MSK1), may induce CREB phosphorylation at Ser^{133} (Shaywitz and Greenberg, 1999; Bengal et al., 2020). Indeed, we observed a substantial increase in the phosphorylation levels of p38 MAPK (2-fold) and AMPK (2-fold), but not in CaMKII, in GAS muscles (Figures 4A–D). On the other hand, we did not observe any significant difference in phosphorylation status of main signaling pathways controlling muscle protein synthesis (i.e., Akt, ERK1/2,

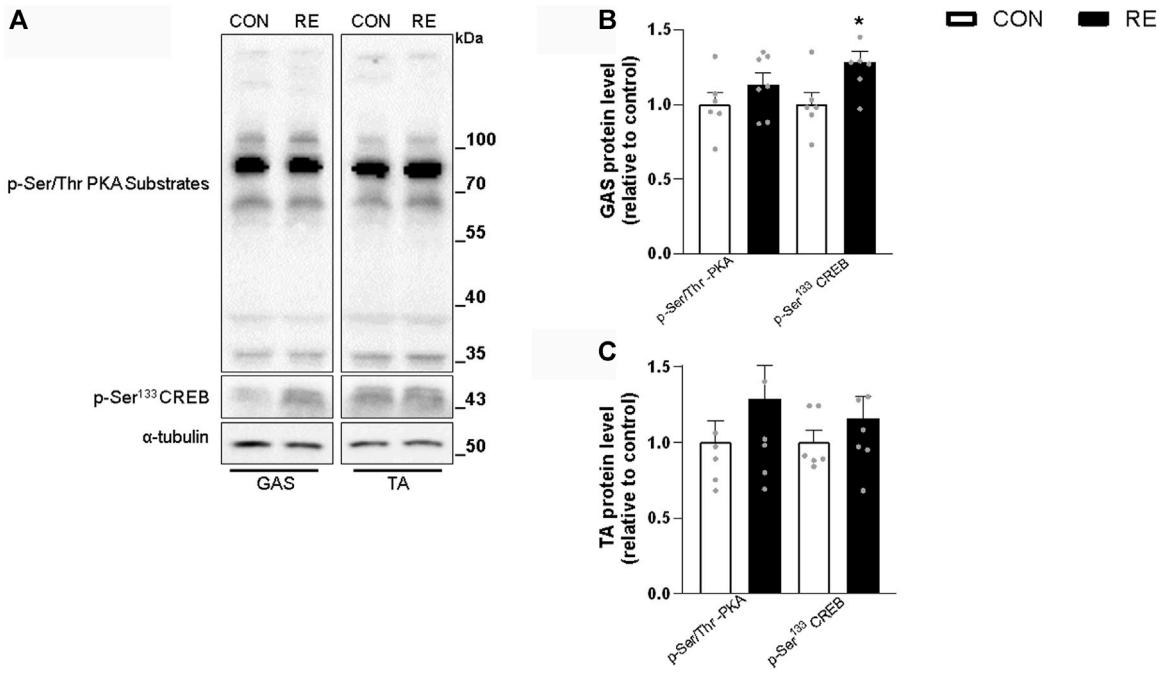


FIGURE 3
Acute effect of a single bout of resistance exercise (RE) on the PKA/CREB signaling pathway in skeletal muscle from mice. **(A)** Representative western blots of phosphorylation levels of PKA substrates and CREB in muscles of exercised mice, immediately (0 h) after the RE session. **(B, C)** Densitometric and statistical analysis of the p-Ser/Thr PKA substrates and p-Ser^{133} CREB protein content in **(B)** *gastrocnemius* (GAS) and **(C)** *tibialis anterior* (TA) muscles. Phosphorylated proteins were normalized to α-tubulin. Data are presented as mean ± SE of 6-7 muscles. (*$p \leq 0.05$ vs CON group, Student's t-test).

and their downstream targets GSK-3 and S6), and protein degradation (i.e., FoxO1 and FoxO3a), when assessed immediately after exercise cessation (Figures 5A–D). Together, these data indicate that a single bout of RE acutely stimulates plasma NE and CREB phosphorylation in muscles, probably via activation of the p38 MAPK and/or AMPK pathways (Thomson et al., 2008).

3.3 β_2-adrenoceptor blockade reduces the CREB-target genes expression in muscles from exercised mice

Because most of the metabolic actions of catecholamines in skeletal muscle are exerted through PKA/CREB signaling pathway (Berdeaux and Stewart, 2012), we hypothesize that the acute increase in plasma NE levels induced by RE would lead to enhanced muscle transcriptional activity of CREB through a β_2-AR-dependent mechanism. To test this hypothesis, we first subjected mice to the maximum voluntary carrying capacity (MVCC) test under β_2-AR blockade with the selective β_2-AR antagonist ICI 118,551 (ICI; 10 mg kg^{-1}, i. p., 30 min prior RE session), in order to evaluate whether ICI pre-treatment would affect mice performance. As shown in Table 3, the mean values of the ICI + RE group were very similar to saline treated-group (41.7 ± 1.1 and 42.8 ± 0.7g, for maximal carrying load; 9.0 ± 1.2 and 10.0 ± 0.7 for number of climbs until exhaustion; 290.69 ± 23.7, and 326.8 ± 24,3 g for training volume; and 16.0 ± 0.9 14.5 ± 1.2min for duration of RE session). Thereafter, we measured the phosphorylation levels of CREB and the mRNA expression of its specific target genes (*i.e.*, *Sik1, Nr4a3, Nr4a1, Ppargc1a* and *Adrb2*), 3 h after the exercise cessation. As shown in Figure 6, *Sik1* (~3-fold), *Nr4a3* (~6-fold) and *Ppargc1a* (~3.5-fold) mRNA levels were upregulated in GAS muscles 3 h after acute RE, even though CREB phosphorylation returned to the basal values (Figures 7E, F). Performing RE under β_2-AR blockage (ICI + RE) resulted in reduced levels of *Sik1* (Figure 6A) and *Nr4a3* mRNA expression (~50%; Figure 6B), when compared with RE alone. On the other hand, ICI + RE did not alter the mRNA expression of *Ppargc1a* (Figure 6D). Interestingly, the *Adrb2* mRNA was not affected by acute RE or ICI + RE (Figure 6E). Thus, our data suggest that acute RE requires the activation of β_2-AR to regulate the muscle expression of specific CREB-target genes, raising the possibility that at least part of this effect might be directly mediated by plasma NOR.

3.4 β_2-AR blockade stimulates the expression of atrophic genes in muscles from exercised mice

Because we have shown that catecholamines and β_2-AR agonists induce anti-catabolic effects on skeletal muscle protein metabolism (Graça et al., 2013; Gonçalves et al., 2019) we next investigated the role of β_2-AR activation in the expression of myokines and UPS- and ALS-related genes 3 h after a single bout of RE. As shown in Figure 7A, the mRNA levels of the negative regulator of muscle mass myostatin (*Mstn*; ~70%), but not the growth factor *igf-1* or the cytokine *Il-6*, was increased by a single bout of RE in GAS muscles. Moreover, acute RE only slightly increased the mRNA expression of

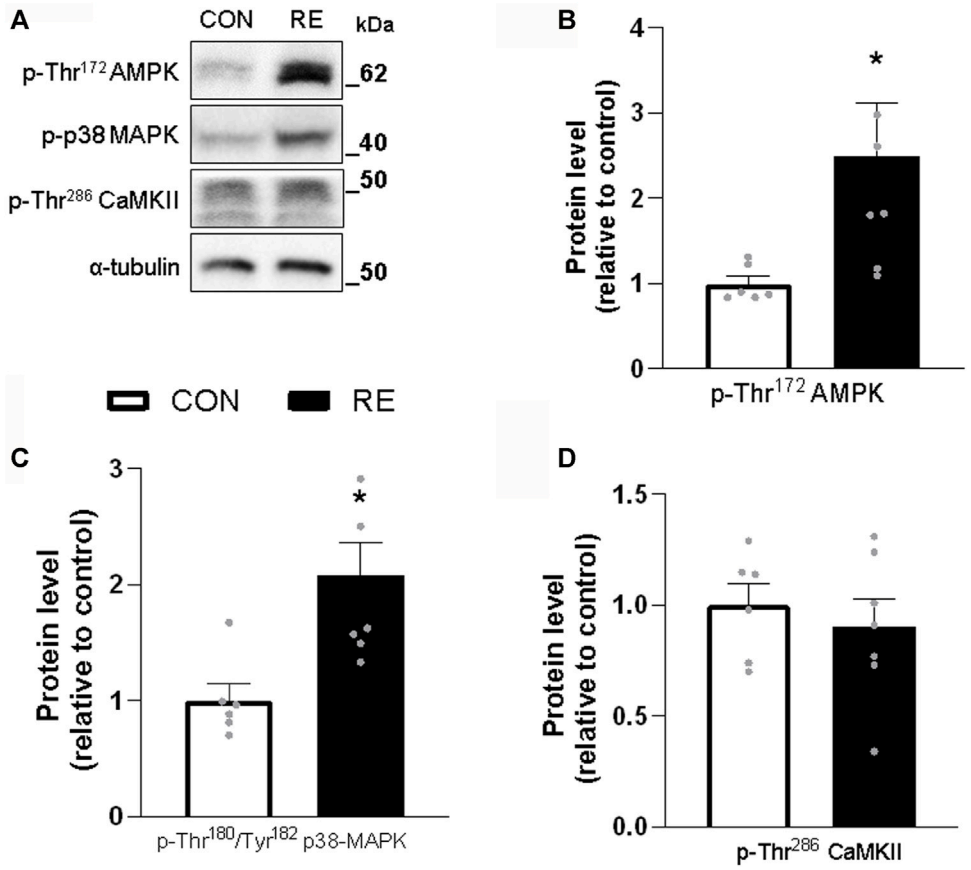


FIGURE 4
Acute effect of a single bout of resistance exercise (RE) on the activation of AMPK, p38 MAPK and CaMKII signaling in muscles of exercised mice. **(A)** Representative western blots of phosphorylation levels of AMPK, p38 MAPK and CaMKII in *gastrocnemius* (GAS) muscles of exercised mice, immediately after (0 h) the RE session. **(B–D)** Densitometric and statistical analysis of the **(B)** p-Thr^{172}AMPK, **(C)** p-Thr$^{180/Tyr182}$ p38 MAPK and **(D)** p-Thr^{286} CaMKII protein content presented in Figure 4A. Phosphorylated proteins were normalized to α-tubulin. Data are presented as mean ± SEM of 6-7 muscles. (*$p \leq .05$ vs CON group, Student's t-test).

Map1lc3b (~30%) and *Gabarapl1* (~50%), an effect that was not observed in *Ctsl* and *Bnip3* (Figures 7B, C). In contrast, the β_2-AR blockade increased the gene expression of *MuRF1/Trim63*, (~3.5-fold), *Atrogin-1/Mafbx32* (~50%), and *Bnip3* (~40%) (Figures 7B, C), as well as the protein content of Atrogin-1 (~80%) (Figure 7E). Importantly, the increase in *Mstn* mRNA expression was higher (~40%) in GAS muscles from ICI + RE, when comparted to RE group (Figure 7A). Because both chronic treatment with β_2-AR agonists and RE training induce a shift in muscle composition from slow to fast phenotype (Mounier et al., 2007), we also measured the mRNA levels of the genes that encodes for myosin heavy chain (MHC) isoforms MHC-I, MHCIIA MHCIIX and MHCIIB (*Myh7, Myh2, Myh1 and Myh4*, respectively). As shown in Figure 7D, neither RE nor ICI + RE affected the mRNA levels of these genes. Altogether, these data indicate that the β_2-AR activation decreases the expression of atrophic genes after a single bout of RE.

4 Discussion

The present study provides molecular evidence that β_2-AR activation during a single bout of RE upregulates the *Nr4a3* gene, a central regulator of the acute RE response, and downregulates the expression of atrophic genes (i.e., *Mstn*, *Murf1/Trim63*, *Atrogin-1/MaFbxo32,* and *Bnip3*) in mouse skeletal muscle after the exercise session. These data provide further evidence that the SNS may be physiologically important to muscle adaptations in response to resistance training. Here, we demonstrated that mice performing a single bout of a ladder climbing-based RE protocol showed a marked increase in plasma NE levels, without significant change in plasma EPI. The increase in plasma catecholamines was expected, because exercise is considered a stressful stimulus that stimulates the sympathoadrenal system to meet the physiological demand of active skeletal muscles (Athanasiou et al., 2023). Although both plasma NE and EPI levels continuously increase as a function of RE intensity (Kraemer and Ratamess, 2005), the plasma NE response is greater when compared to EPI (Greiwe et al., 1999). Similar to plasma catecholamines, muscle sympathetic nerve activity (MSNA) gradually increases in proportion to the rise in exercise intensity (Katayama and Saito, 2019). More important, we and others have recently demonstrated that the SNS directly innervates neuromuscular junctions and regulates skeletal muscle metabolism, fiber type composition, and cross-sectional area in normal conditions (Khan et al., 2016; Rodrigues et al., 2019). In the present study,

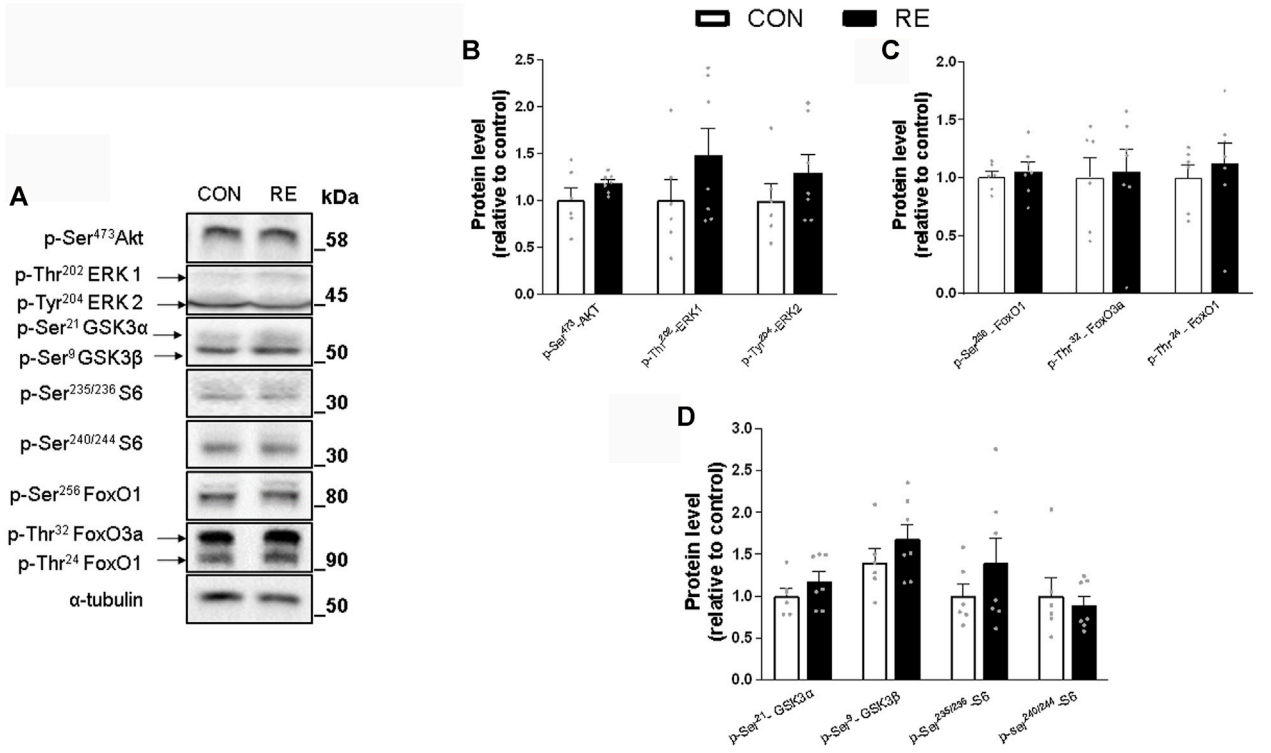


FIGURE 5
Acute effect of a single bout of resistance exercise (RE) on the Insulin/IGF-1 signaling in muscles of exercised mice. **(A)** Representative Western blot of phosphorylation levels of Akt, ERK1/2, GSK3, S6, FoxO1 and FoxO3 in *gastrocnemius* (GAS) muscles of exercised mice, immediately after (0 h) the RE session. **(B–D)** Densitometric and statistical analysis of the **(B)** Akt and ERK/12; **(C)** FoxO1 and FoxO3a; and **(D)** GSK3 and S6 protein content. Phosphorylated proteins were normalized to α-tubulin. Data are presented as mean ± SEM of 6-7 muscles. (*$p \leq 0.05$ vs CON group, Student's t-test).

TABLE 3 Body mass and maximum voluntary carrying capacity (MVCC) data.

Parameter	CON	RE	RE + ICI
Body mass (g)	21.6 ± 0.7	21.8 ± 1.49	21.1 ± 0.6
Maximal load (g)	43.7 ± 1.3	41.7 ± 1.1	42.8 ± 0.7
Number of climbs	-	9.0 ± 1.2	10.0 ± 0.7
Training volume (g)	-	290.69 ± 23.7	326.8 ± 24.3
Protocol time (min)	-	16.0 ± 0.9	14.5 ± 1.2

All values are presented as means ± standard error of the mean; CON: control group; RE: resistance exercise group; ICI + RE: resistance exercise under β_2-AR, blockade group.

however, the increase in muscle content of NE was not observed after the RE session, most likely due to NE diffusion from synapses (mainly from active muscles) into the plasma (Rowell and O'Leary, 1990). Indeed, plasma NE concentration is greatly influenced by the rate of NE diffusion. Therefore, it cannot be excluded that an increase of NE release directly into muscle may have occurred during the RE. The measurement of muscle NE turnover (Graça et al., 2013) and muscle sympathetic denervation (Silveira et al., 2014) would be necessary to determine the direct contribution of muscle sympathetic activity to the molecular changes induced by acute RE.

Independently of the systemic or local origin of NE, we have pieces of evidence that SNS stimulates muscle cells in response to RE. The finding in GAS muscle that a single bout of RE increased the phosphorylation levels of CREB, a well-established target of PKA, and its specific target genes (i.e., *Sik1*, *Nr4a3*, and *Ppargc1a*), suggests that the canonic β_2-AR/PKA/CREB signaling pathway were stimulated. Consistently, the β_2-AR blockage downregulated the *Sik1* and *Nr4a3* gene expression in GAS muscles from exercised mice. Similar findings were reported by Bruno et al. (Bruno et al., 2014) showing that the expression of *Sik1* mRNA was markedly induced by a high-intensity exercise, and this response was prevented by the pre-treatment with propranolol, a pan *ß*-AR antagonist (Bruno et al., 2014; Goode et al., 2016). However, a few studies have investigated the role of the protein kinase SIK-1 on skeletal muscle physiology. Berdeaux et al. (Berdeaux et al., 2007) proposed that, under adrenergic stimulation with the *ß*-adrenergic agonist isoproterenol, CREB activates the myogenic

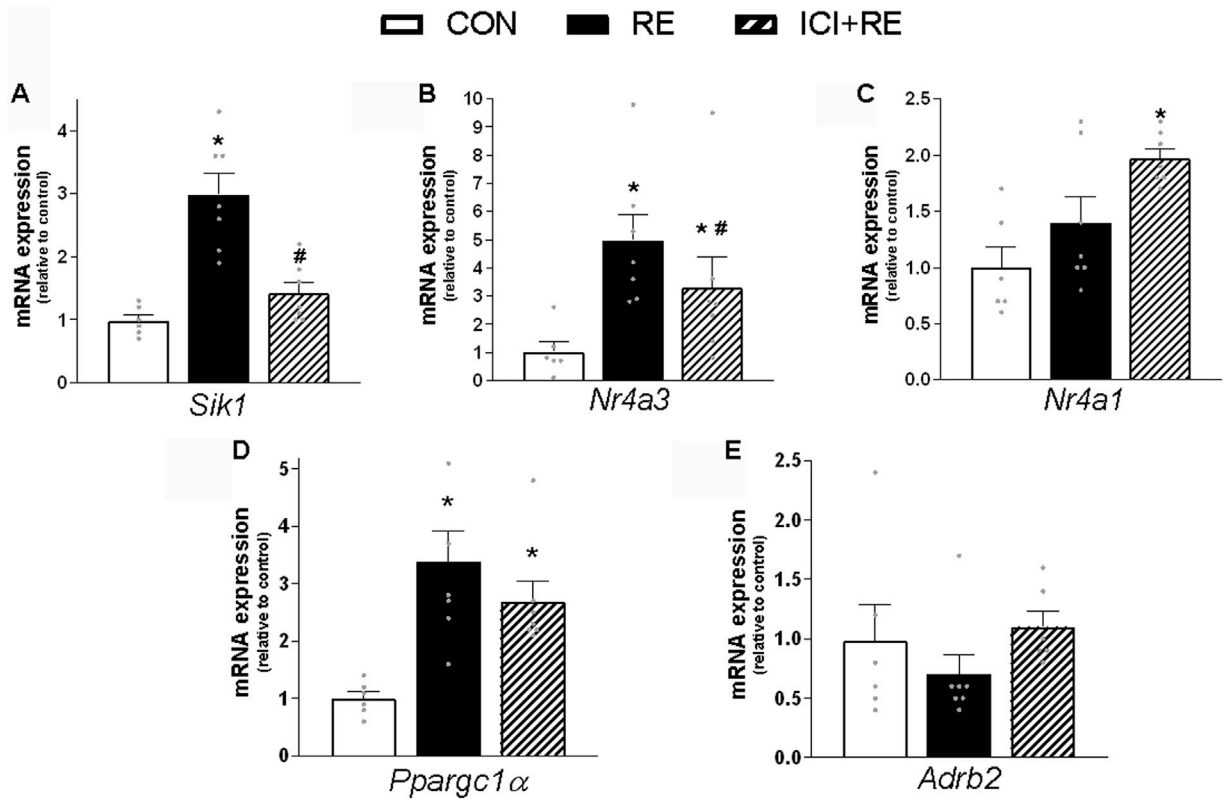


FIGURE 6
Effect of the β_2-AR blockade on CREB-target genes expression in skeletal muscle of exercised mice. Three hours (3 h) after the exercise session, the **(A)** *Sik1;* **(B)** *Nr4a3;* **(C)** *Nr4a1;* **(D)** *Ppargc1α* and **(E)** *Adrb2* mRNA levels were analyzed in *gastrocnemius* (GAS) muscles from exercised mice pre-treated with β_2-AR antagonist ICI 118,551 (single dose - 10 mg kg^{-1}, i. p., 30 min prior RE). Data are presented as mean ± SEM of 6-7 muscles. (*$p \leq 0.05$ vs CON group, #$p < 0.05$ vs RE group, bidirectional AVONA test).

program by increasing the amount of SIK-1 in C2C12 cells and adult skeletal muscles. When activated (dephosphorylated form), SIK-1 phosphorylates class II histone deacetylases (HDACs) and indirectly promotes the expression of myocyte enhancer factor 2 (MEF2) target genes (Berdeaux et al., 2007). Accordantly, the overexpression of SIK1 in muscle cells induces nuclear export of HDAC5 and increases MEF-2C transcriptional activity *in vitro* (Takemori et al., 2009). Despite these data, there is no evidence that SIK-1 regulates muscle protein metabolism in response to exercise.

In contrast to our data, Goode et al. (2016) suggested that the increase in *Nr4a3* mRNA after endurance exercise is independent on *ß*-AR signaling, since its expression was not attenuated by the treatment with the *ß*-AR antagonist propranolol. These conflicting results may be due to differences in the experimental design, including pharmacological treatment (selective *versus* non-selective β_2-AR antagonist) and type of exercise bout (endurance exercise *versus* RE). Despite that, recent evidences have shown that the *Nr4a3* gene was robustly induced by a novel RE model in mice (Cui et al., 2020) and by acute RE in human skeletal muscle (Nader et al., 2014). By using gene ontology to highlight pathways activated by inactivity, aerobic *versus* resistance, and acute *versus* chronic exercise, Pillon et al. (2020) identified *Nr4a3* as the most exercise- and inactivity-responsive gene, and determined its role in the regulation of mitochondrial function and glucose uptake in response to electrical pulse stimulation in human myotubes *in vitro*. Additionally, it has been shown that transgenic overexpression of NR4A3 promotes skeletal muscle hypertrophy, oxidative phenotype, and vascularization in mice (Goode et al., 2016). Although it has already been shown that acute exercise (Goode et al., 2016; Cui et al., 2020) and β_2-AR stimulation (Berdeaux et al., 2007; Pearen et al., 2009) alone may induce the expression of *Sik1* and *Nr4a3* mRNA, to our knowledge, this is the first study to demonstrate that the pre-treatment with the selective β_2-AR antagonist downregulates the expression of both genes in muscles from exercised mice. Further studies are needed to reveal the specific role of *Nr4a3* and *Sik-1* expression in mediating the effects of β_2-AR stimulation on muscle protein metabolism in response to chronic RE training.

The reason for differences between TA and GAS muscles in response to acute RE cannot be accounted for in the present study, but exercised muscles are either under the influence of extrinsic (*e.g.*, neural and hormonal) and intrinsic (*e.g.*, mechanical and metabolic) factors that activate/repress several intracellular signaling pathways (Egan and Zierath, 2013). For example, the finding that CREB phosphorylation was induced only in GAS muscles reinforces the hypothesis that acute RE increased the sympathetic activityprobably by a metaborreflex-induced mechanism (Fisher et al., 2015), since GAS muscle, but not TA, is highly recruited during climbing movements (Lourenço et al., 2020). On the other hand, the fact that the expression of *Ppargc1a* mRNA induced by RE was not affected by β_2-AR blockage raise the possibility that the expression of this transcription

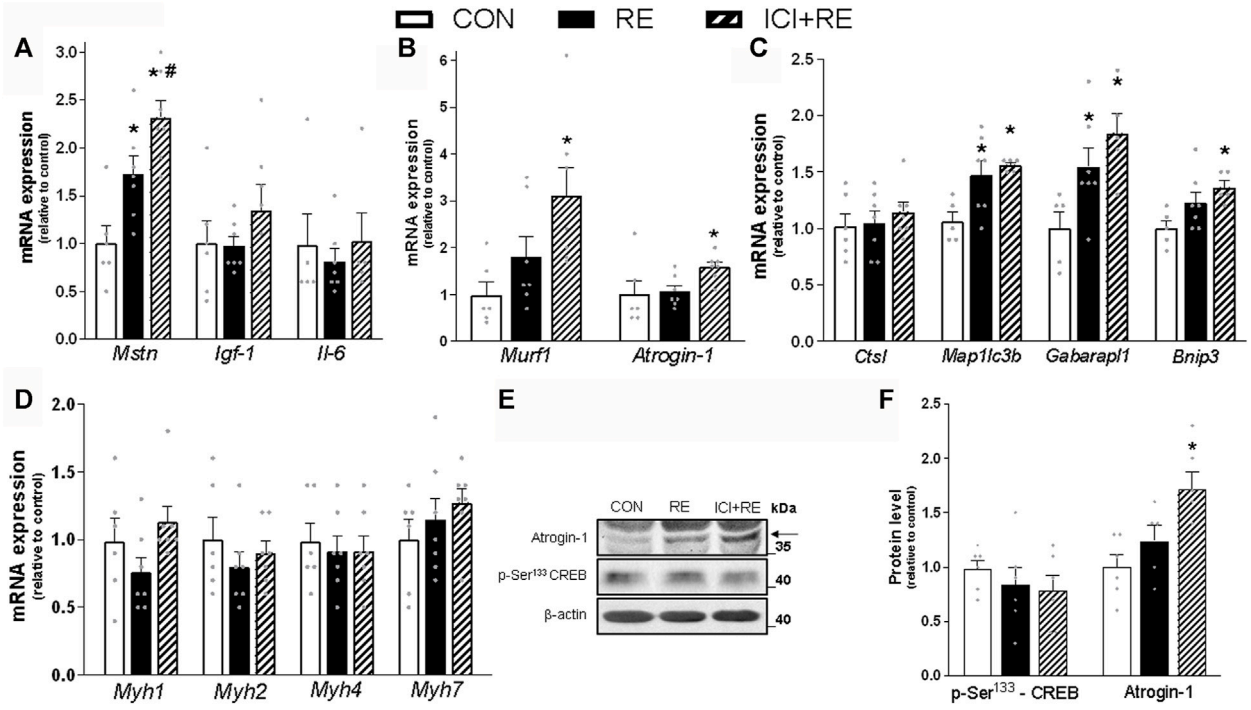


FIGURE 7
Effect of the β_2-AR blockade on the mRNA levels of genes involved in metabolism and plasticity in muscles of exercised mice. Three hours (3 h) after the exercise session, the **(A)** *Mstn*, *igf-1* and *Il-6* (myokines); **(B)** *Murf1* and *Atrogin-1* (atrophy-related genes); and **(C)** *Ctsl*, *Map1lc3b*, *Gabarapl1* and *Bnip3* (autophagy-related genes); **(D)** *Myh1*, *Myh2*, *Myh4* and *Myh7* mRNA levels were analyzed in *gastrocnemius* (GAS) muscles of exercised mice pre-treated with β_2-AR antagonist ICI 118,551 (single dose; 10 mg kg^{-1}, i. p., 30 min prior RE). **(E)** Representative western blots of phosphorylation levels of CREB and protein content of Atrogin-1 in gastrocnemius (GAS) muscles of exercised mice, three hours (3 h) after the exercise session. **(F)** Densitometric and statistical analysis of the protein content presented in **(E)**. Phosphorylated proteins were normalized to β-actin. Data are presented as mean ± SEM of 6-7 muscles. (*$p \leq 0.05$ vs CON group, #$p < 0.05$ vs RE group, bidirectional AVONA test).

co-activator is under control of intrinsic signals, such as increased intracellular calcium concentration $[Ca^{2+}]_i$, AMP:ATP ratio and mechanical tension, among others (Egan and Zierath, 2013). Accordingly, these signals may trigger the activation of protein kinases involved in several metabolic processes including AMPK, CaMKII, and p38 MAPK. In agreement with this notion, we demonstrated that the phosphorylation levels of AMPK and p38 MAPK, but not CaMKII, increased in GAS muscle immediately after acute RE. It is important to mention that these kinases seem to be activated by an intensity-dependent manner and may stimulate *Ppargc1a* gene transcription by different regulators, such as activating transcription factor2 (ATF2), myocyte enhancer factor 2 (MEF2), CREB, and HDACs (Wojtaszewski et al., 2000; Rose et al., 2006; Akimoto et al., 2008; Egan and Zierath, 2013). Indeed, it has been shown that p38 MAPK can phosphorylate and activate the ATF2 transcription factor, whereas AMPK can directly phosphorylate CREB during exercise, upregulating *Ppargc1a* mRNA (Akimoto et al., 2008; Thomson et al., 2008). Taken together, these data suggest that acute RE may upregulate *Ppargc1a* expression by multiple muscle intrinsic signals in a β_2-AR-independent manner.

As previously reported (Silvestre et al., 2017), our ladder climbing-based progressive RE increased serum corticosterone (CORT) levels immediately after exercise, and probably reduced insulin (INS) secretion in exercised mice (Raastad et al., 2000; Kraemer and Ratamess, 2005). During catabolic conditions, such as fasting and type 1 diabetes mellitus, the high levels of glucocorticoids associated with low levels of circulating insulin drive the activation of muscle proteolysis by UPS and ALS in order to support liver gluconeogenesis and the energy requirements of the organism (Hu et al., 2009; Graça et al., 2013). In fact, Hu et al. (2009) have demonstrated that both impaired INS signaling and increased endogenous glucocorticoids are required to stimulate muscle proteolysis by UPS. Thus, reduced activity of the INS/Akt signaling leads to a marked increase in atrophic genes and muscle atrophy via the transcriptional activity of FoxO members (*e.g.*, FoxO1 and FoxO3a) (Schiaffino and Mammucari, 2011). Due to the catabolic nature of the acute exercise, it has been shown that the rate of overall protein degradation and the induction of these atrophic genes also increases in human skeletal muscle immediately after the acute RE, likely to prevent the accumulation of damaged proteins and organelles (Phillips et al., 1997; Louis et al., 2007). However, the effect of a single bout of progressive RE-induced acute molecular changes in rodent skeletal muscle remain poorly explored. The present study shows that a single bout of RE performed until exhaustion did not affect the phosphorylation and activation/inactivation status of the major signaling pathways controlling muscle protein synthesis (*i.e.*, Akt, ERK1/2, and their downstream targets GSK-3 and mTOR), and protein degradation (*i.e.*, FoxO), when assessed immediately after exercise session. These data are controversial because published data have not been consistent on whether these signaling are increased or unaffected in response to acute RE (Bolster et al., 2003; Hamilton et al., 2010; Kido et al., 2016; McIntosh et al., 2023). Again, these conflicting results may be due to differences in the experimental approaches,

including exercise protocol, species (rodents *versus* humans), and time point of sample collecting after the exercise bout, among others.

It is well known that the SNS also contributes to the establishment of a catabolic state during acute exercise since catecholamines stimulates the catabolism of glycogen and intramuscular triglyceride through β_2-AR// PKA/CREB signaling in skeletal muscle (Egan and Zierath, 2013; Bruno et al., 2014). Conversely, we and others have consistently shown that both catecholamines and β_2-AR agonists exert anabolic and anticatabolic actions on protein metabolism, contributing to the maintenance of skeletal muscle mass under basal and catabolic conditions (Navegantes et al., 1999; Baviera et al., 2008; Graça et al., 2013; Khan et al., 2016; Gonçalves et al., 2019; Rodrigues et al., 2019). Thus, we hypothesized that the activation of β_2-ARs during acute RE could inhibit the expression of atrophic genes in the skeletal muscle of exercised mice. Accordingly, we demonstrate that performing acute RE under β_2-AR blockade upregulates the expression of the Ub-ligases *Murf1/Trim63* and *Atrogin-1/Mafbx32*. Although the exact molecular mechanisms cannot be accounted for in the present study, treatment with β_2-agonists has been suggested to inhibit atrophic gene expression by stimulating Akt/FoxO signaling pathway (Gonçalves et al., 2019; Lynch and Ryall, 2008). Our findings are in partial agreement with other studies showing that chemical (Baviera et al., 2008) or surgical (Graça et al., 2013) sympathectomy exacerbates atrophic genes during type I diabetes and fasting, respectively, apparently by a further decrease in Akt stimulation. An alternative possibility to the suppressive action of SNS in atrophic genes during RE is that cAMP/PKA signaling could mediate such an effect. In agreement with this notion, we have recently shown that muscle-specific overexpression of PKI (PKA inhibitor peptide) decreased the phosphorylation levels of CREB and upregulated the transcriptional activity of FoxO and the mRNA expression of *Atrogin-1* and *MuRF1*, resulting in myofiber atrophy (Silveira et al., 2020). More important, the muscle-specific activating of PKA by the overexpression of PKA catalytic subunit suppressed FoxO transcriptional activity by multiple mechanisms *in vivo*, in addition to FoxO phosphorylation, acetylation, and nuclear export (Silveira et al., 2020), raising the possibility that this cAMP/PKA/FoxO signaling may have mediate this anti-catabolic action. Further studies are required to confirm this hypothesis.

An interesting finding of this study was that performing acute RE under β_2-AR blockade amplified the expression of *Mstn* mRNA in exercised muscles, suggesting that the β_2-AR signaling restrains *Mstn* overexpression during RE. Since it has been shown that the expression of a constitutively active form of FoxO1 may upregulate *Mstn* mRNA in differenciated C2C12 myotubes (Allen and Unterman, 2007), it is reasonable to speculate that prevention of the activation of PKA/CREB signaling by ICI in the muscles of exercised mice could exacerbate FoxO activity and consequently *Mstn* expression. Additionally, it is also possible that the downregulation of *N4a3* expression by β_2-AR blockade could further upregulate the *Mstn* mRNA levels in muscles of exercised mice. This hypothesis is based on findings that reduced endogenous *Nr4a3* mRNA levels induced by stable expression of a NOR-1 (*i.e.,* NR4A3) small interfering RNA in C2C12 cells led to a dramatic upregulation of *Mstn* mRNA expression, whereas *NR4a3* overexpression induced by an expression vector (pSG5-NOR-1) repressed *Mstn* promoter activity and gene expression (Pearen et al., 2009; Goode et al., 2016). Although these findings suggest that β_2-AR/PKA/CREB signaling may contribute to the adaptive anabolic pathways in response to RE, further experiments are needed to confirm all these hypotheses.

In summary, the present data suggest that β_2-AR stimulation during acute RE upregulates the expression of the hypertrophic gene *Nr4a3* and restrains the atrophic genes *Mstn*, *Murf1/Trim63*, *Atrogin-1/MaFbxo32* and *Bnip3* in skeletal muscle. These effects may be physiologically important for preventing excessive protein breakdown during muscle contractions and for establishing the anabolic state observed during recovery from acute RE, which may contribute, at least in part, to the muscle adaptations in response to regular RE training.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary materials, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by the Commission of Ethics in Animal Research from Ribeirão Preto Medical School of the University of São Paulo. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

RA-S: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing–original draft, Writing–review and editing. GZ: Investigation, Methodology, Writing–review and editing. NL: Investigation, Methodology, Supervision, Writing–review and editing. AS: Investigation, Writing–review and editing, Conceptualization, Formal Analysis, Validation. LH: Investigation, Methodology, Writing–review and editing. DG: Conceptualization, Supervision, Writing–review and editing, Data curation, Investigation, Methodology. IK: Conceptualization, Supervision, Writing–review and editing, Data curation. LN: Conceptualization, Data curation, Supervision, Writing–review and editing. WS: Conceptualization, Data curation, Investigation, Methodology, Supervision, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by grants to NL (FAPESP 2019/10420-3); DG (FAPEMIG APQ-01268-21 and APQ-02960-22); IK (FAPESP 2018/10089-2); and LN (PQ CNPq: 302396/2022-5; CAPES/PROEX).

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.

Publisher's note

References

Akimoto, T., Li, P., and Yan, Z. (2008). Functional interaction of regulatory factors with the *Pgc-1* α promoter in response to exercise by *in vivo* imaging. *Am. J. Physiology-Cell Physiology* 295, C288–C292. doi:10.1152/ajpcell.00104.2008

Allen, D. L., and Unterman, T. G. (2007). Regulation of myostatin expression and myoblast differentiation by FoxO and SMAD transcription factors. *Am. J. Physiology-Cell Physiology* 292, C188–C199. doi:10.1152/ajpcell.00542.2005

Altarejos, J. Y., and Montminy, M. (2011). CREB and the CRTC co-activators: sensors for hormonal and metabolic signals. *Nat. Rev. Mol. Cell Biol.* 12, 141–151. doi:10.1038/nrm3072

Athanasiou, N., Bogdanis, G. C., and Mastorakos, G. (2023). Endocrine responses of the stress system to different types of exercise. *Rev. Endocr. Metabolic Disord.* 24, 251–266. doi:10.1007/s11154-022-09758-1

Aweida, D., and Cohen, S. (2021). Breakdown of filamentous myofibrils by the UPS–step by step. *Biomolecules* 11, 110. doi:10.3390/biom11010110

Azevedo Voltarelli, V., Coronado, M., Gonçalves Fernandes, L., Cruz Campos, J., Jannig, P. R., Batista Ferreira, J. C., et al. (2021). β2-Adrenergic signaling modulates mitochondrial function and morphology in skeletal muscle in response to aerobic exercise. *Cells* 10, 146. doi:10.3390/cells10010146

Baviera, A. M., Zanon, N. M., Navegantes, L. C. C., Migliorini, R. H., and Kettelhut, I. C. (2008). Chemical sympathectomy further increases muscle protein degradation of acutely diabetic rats. *Muscle Nerve* 38, 1027–1035. doi:10.1002/mus.21018

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

Berdeaux, R., Goebel, N., Banaszynski, L., Takemori, H., Wandless, T., Shelton, G. D., et al. (2007). SIK1 is a class II HDAC kinase that promotes survival of skeletal myocytes. *Nat. Med.* 13, 597–603. doi:10.1038/nm1573

Berdeaux, R., and Stewart, R. (2012). cAMP signaling in skeletal muscle adaptation: hypertrophy, metabolism, and regeneration. *Am. J. Physiology-Endocrinology Metabolism* 303, E1–E17. doi:10.1152/ajpendo.00555.2011

Bolster, D. R., Kubica, N., Crozier, S. J., Williamson, D. L., Farrell, P. A., Kimball, S. R., et al. (2003). Immediate response of mammalian target of rapamycin (mTOR)-Mediated signalling following acute resistance exercise in rat skeletal muscle. *J. Physiol.* 553, 213–220. doi:10.1113/jphysiol.2003.047019

Bruno, N. E., Kelly, K. A., Hawkins, R., Bramah-Lawani, M., Amelio, A. L., Nwachukwu, J. C., et al. (2014). Creb coactivators direct anabolic responses and enhance performance of skeletal muscle. *EMBO J.* 33, 1027–1043. doi:10.1002/embj.201386145

Cairns, S. P., and Borrani, F. (2015). β-Adrenergic modulation of skeletal muscle contraction: key role of excitation-contraction coupling. *J. Physiol.* 593, 4713–4727. doi:10.1113/JP270909

Cui, D., Drake, J. C., Wilson, R. J., Shute, R. J., Lewellen, B., Zhang, M., et al. (2020). A novel voluntary weightlifting model in mice promotes muscle adaptation and insulin sensitivity with simultaneous enhancement of autophagy and mTOR pathway. *FASEB J.* 34, 7330–7344. doi:10.1096/fj.201903055R

Durlo, F. v., Castro, M., Elias, L. L. K., and Antunes-Rodrigues, J. (2004). Interaction of prolactin, ANPergic, oxytocinergic and adrenal systems in response to extracellular volume expansion in rats. *Exp. Physiol.* 89, 541–548. doi:10.1113/expphysiol.2004.027243

Egan, B., and Sharples, A. P. (2023). Molecular responses to acute exercise and their relevance for adaptations in skeletal muscle to exercise training. *Physiol. Rev.* 103, 2057–2170. doi:10.1152/physrev.00054.2021

Egan, B., and Zierath, J. R. (2013). Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell metab.* 17, 162–184. doi:10.1016/j.cmet.2012.12.012

Elfellah, M., Dalling, R., Kantola, I., and Reid, J. (1989). Beta-adrenoceptors and human skeletal muscle characterisation of receptor subtype and effect of age. *Br. J. Clin. Pharmacol.* 27, 31–38. doi:10.1111/j.1365-2125.1989.tb05332.x

Fatouros, I., Chatzinikolaou, A., Paltoglou, G., Petridou, A., Avloniti, A., Jamurtas, A., et al. (2010). Acute resistance exercise results in catecholaminergic rather than hypothalamic–pituitary–adrenal axis stimulation during exercise in young men. *Stress* 13, 461–468. doi:10.3109/10253891003743432

Figueiredo, V. C., and Markworth, J. F. (2015). Mechanisms of protein synthesis activation following exercise: new pieces to the increasingly complex puzzle. *J. Physiol.* 593, 4693–4695. doi:10.1113/JP271431

Fisher, J. P., Young, C. N., and Fadel, P. J. (2015). "Autonomic adjustments to exercise in humans," in *Comprehensive physiology* (New Jersey, United States: Wiley), 475–512. doi:10.1002/cphy.c140022

Frajacomo, F. T., Kannen, V., Deminice, R., Geraldino, T. H., Ppereira-da-Silva, G., Uyemura, S. A., et al. (2015). Aerobic training activates interleukin 10 for colon anticarcinogenic effects. *Med. Sci. Sports Exerc* 47, 1806–1813. doi:10.1249/MSS.0000000000000623

Garofalo, M. A. R., Kettelhut, I. C., Roselino, J. S., and Migliorini, R. H. (1996). Effect of acute cold exposure on norepinephrine turnover rates in rat white adipose tissue. *J. Auton. Nerv. Syst.* 60, 206–208. doi:10.1016/0165-1838(96)00037-9

Gonçalves, D. A., Silveira, W. A., Manfredi, L. H., Graça, F. A., Armani, A., Bertaggia, E., et al. (2019). Insulin/IGF1 signalling mediates the effects of β2 -adrenergic agonist on muscle proteostasis and growth. *J. cachexia, sarcopenia muscle* 10, 455–475. doi:10.1002/jcsm.12395

Gonçalves, D. A. P., Silveira, W. A., Lira, E. C., Graça, F. A., Paula-Gomes, S., Zanon, N. M., et al. (2012). Clenbuterol suppresses proteasomal and lysosomal proteolysis and atrophy-related genes in denervated rat soleus muscles independently of Akt. *Am. J. physiology. Endocrinol. metabolism* 302, E123–E133. doi:10.1152/ajpendo.00188.2011

Goode, J. M., Pearen, M. A., Tuong, Z. K., Wang, S.-C. M., Oh, T. G., Shao, E. X., et al. (2016). The nuclear receptor, nor-1, induces the physiological responses associated with exercise. *Mol. Endocrinol.* 30, 660–676. doi:10.1210/me.2015-1300

Goto, K., Takahashi, K., Yamamoto, M., and Takamatsu, K. (2008). Hormone and recovery responses to resistance exercise with slow movement. *J. Physiological Sci.* 58, 7–14. doi:10.2170/physiolsci.RP003107

Graça, F. A., Gonçalves, D. A. P., Silveira, W. A., Lira, E. C., Chaves, V. E., Zanon, N. M., et al. (2013). Epinephrine depletion exacerbates the fasting-induced protein breakdown in fast-twitch skeletal muscles. *Am. J. Physiol. Endocrinol. Metab.* 305, 1483–1494. doi:10.1152/ajpendo.00267.2013

Graham, Z. A., Lavin, K. M., O'Bryan, S. M., Thalacker-Mercer, A. E., Buford, T. W., Ford, K. M., et al. (2021). Mechanisms of exercise as a preventative measure to muscle wasting. *Am. J. Physiology-Cell Physiology* 321, C40–C57. doi:10.1152/ajpcell.00056.2021

Greiwe, J. S., Hickner, R. C., Shah, S. D., Cryer, P. E., and Holloszy, J. O. (1999). Norepinephrine response to exercise at the same relative intensity before and after endurance exercise training. *J. Appl. Physiol.* 86, 531–535. doi:10.1152/jappl.1999.86.2.531

Grumati, P., Coletto, L., Schiavinato, A., Castagnaro, S., Bertaggia, E., Sandri, M., et al. (2011). Physical exercise stimulates autophagy in normal skeletal muscles but is detrimental for collagen VI-deficient muscles. *Autophagy* 7, 1415–1423. doi:10.4161/auto.7.12.17877

Hamilton, D. L., Philp, A., MacKenzie, M. G., and Baar, K. (2010). A limited role for PI(3,4,5)P3 regulation in controlling skeletal muscle mass in response to resistance exercise. *PLoS One* 5, e11624. doi:10.1371/journal.pone.0011624

Harcourt, L. J., Schertzer, J. D., Ryall, J. G., and Lynch, G. S. (2007). Low dose formoterol administration improves muscle function in dystrophic mdx mice without increasing fatigue. *Neuromuscul. Disord. NMD* 17, 47–55. doi:10.1016/j.nmd.2006.08.012

Hornberger, T. A., and Farrar, R. P. (2004). Physiological hypertrophy of the FHL muscle following 8 Weeks of progressive resistance exercise in the rat. *Can. J. Appl. Physiology* 29, 16–31. doi:10.1139/h04-002

Hu, Z., Wang, H., Lee, I. H., Du, J., and Mitch, W. E. (2009). Endogenous glucocorticoids and impaired insulin signaling are both required to stimulate muscle wasting under pathophysiological conditions in mice. *J. Clin. Investigation* 119, 3059–3069. doi:10.1172/JCI38770

Katayama, K., and Saito, M. (2019). Muscle sympathetic nerve activity during exercise. *J. Physiological Sci.* 69, 589–598. doi:10.1007/s12576-019-00669-6

Khan, M. M., Lustrino, D., Silveira, W. A., Wild, F., Straka, T., Issop, Y., et al. (2016). Sympathetic innervation controls homeostasis of neuromuscular junctions in health and disease. *Proc. Natl. Acad. Sci.* 113, 746–750. doi:10.1073/pnas.1524272113

Kido, K., Ato, S., Yokokawa, T., Makanae, Y., Sato, K., and Fujita, S. (2016). Acute resistance exercise-induced IGF1 expression and subsequent GLUT4 translocation. *Physiol. Rep.* 4, e12907. doi:10.14814/phy2.12907

Kim, J., Grotegut, C. A., Wisler, J. W., Li, T., Mao, L., Chen, M., et al. (2018). β-arrestin 1 regulates β2-adrenergic receptor-mediated skeletal muscle hypertrophy and contractility. *Skelet. Muscle* 8, 39. doi:10.1186/s13395-018-0184-8

Kim, J., Yang, G., Kim, Y., Kim, J., and Ha, J. (2016). AMPK activators: mechanisms of action and physiological activities. *Exp. Mol. Med.* 48, 2244–e312. doi:10.1038/emm.2016.16

Kim, Y. S., and Sainz, R. D. (1992). Beta-adrenergic agonists and hypertrophy of skeletal muscles. *Life Sci.* 50, 397–407. doi:10.1016/0024-3205(92)90374-x

Kline, W. O., Panaro, F. J., Yang, H., and Bodine, S. C. (2007). Rapamycin inhibits the growth and muscle-sparing effects of clenbuterol. *J. Appl. physiology (Bethesda, Md, 1985)* 102, 740–747. doi:10.1152/japplphysiol.00873.2006

Koopman, R., Gehrig, S. M., Léger, B., Trieu, J., Walrand, S., Murphy, K. T., et al. (2010). Cellular mechanisms underlying temporal changes in skeletal muscle protein synthesis and breakdown during chronic {beta}-adrenoceptor stimulation in mice. *J. physiology* 588, 4811–4823. doi:10.1113/jphysiol.2010.196725

Kraemer, W. J., and Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. *Sports Med. Auckl. N.Z.)* 35, 339–361. doi:10.2165/00007256-200535040-00004

Louis, E., Raue, U., Yang, Y., Jemiolo, B., and Trappe, S. (2007). Time course of proteolytic, cytokine, and myostatin gene expression after acute exercise in human skeletal muscle. *J. Appl. Physiol.* 103, 1744–1751. doi:10.1152/japplphysiol.00679.2007

Lourenço, Í., Krause Neto, W., Santos Portella Amorim, L., Moraes Munhoz Ortiz, V., Lopes Geraldo, V., Henrique da Silva Ferreira, G., et al. (2020). Muscle hypertrophy and ladder-based resistance training for rodents: a systematic review and meta-analysis. *Physiol. Rep.* 8, 1–25. doi:10.14814/phy2.14502

Lynch, G. S., and Ryall, J. G. (2008). Role of beta-adrenoceptor signaling in skeletal muscle: implications for muscle wasting and disease. *Physiol. Rev.* 88, 729–767. doi:10.1152/physrev.00028.2007

Macedo, A. G., Almeida, T. A. F., Massini, D. A., De Paula, V. F., De Oliveira, D. M., and Pessôa Filho, D. M. (2023). Effects of exercise training on glucocorticoid-induced muscle atrophy: literature review. *Steroids* 195, 109240. doi:10.1016/j.steroids.2023.109240

Mathur, S., Brooks, D., and Carvalho, C. R. F. (2014). Structural alterations of skeletal muscle in copd. *Front. Physiology* 5, 104. doi:10.3389/fphys.2014.00104

McIntosh, M. C., Sexton, C. L., Godwin, J. S., Ruple, B. A., Michel, J. M., Plotkin, D. L., et al. (2023). Different resistance exercise loading paradigms similarly affect skeletal muscle gene expression patterns of myostatin-related targets and mTORC1 signaling markers. *Cells* 12, 898. doi:10.3390/cells12060898

Mersmann, H. J. (1998). Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. *J. Animal Sci.* 76, 160–172. doi:10.2527/1998.761160x

Milan, G., Romanello, V., Pescatore, F., Armani, A., Paik, J.-H., Frasson, L., et al. (2015). Regulation of autophagy and the ubiquitin–proteasome system by the FoxO transcriptional network during muscle atrophy. *Nat. Commun.* 6, 6670. doi:10.1038/ncomms7670

Miyazaki, M., and Takemasa, T. (2017). TSC2/Rheb signaling mediates ERK-dependent regulation of mTORC1 activity in C2C12 myoblasts. *FEBS Open Bio* 7, 424–433. doi:10.1002/2211-5463.12195

Mounier, R., Cavalié, H., Lac, G., and Clottes, E. (2007). Molecular impact of clenbuterol and isometric strength training on rat EDL muscles. *Pflugers Archiv Eur. J. physiology* 453, 497–507. doi:10.1007/s00424-006-0122-1

Nader, G. A., von Walden, F., Liu, C., Lindvall, J., Gutmann, L., Pistilli, E. E., et al. (2014). Resistance exercise training modulates acute gene expression during human skeletal muscle hypertrophy. *J. Appl. Physiol.* 116, 693–702. doi:10.1152/japplphysiol.01366.2013

Navegantes, L. C. C., Resano, N. M. Z., Migliorini, R. H., and Kettelhut, I. C. (1999). Effect of guanethidine-induced adrenergic blockade on the different proteolytic systems in rat skeletal muscle. *Am. J. Physiology-Endocrinology Metabolism* 277, E883–E889. doi:10.1152/ajpendo.1999.277.5.E883

Osman, A. A., Pendergrass, M., Koval, J., Maezono, K., Cusi, K., Pratipanawatr, T., et al. (2000). Regulation of MAP kinase pathway activity *in vivo* in human skeletal muscle. *Am. J. Physiology-Endocrinology Metabolism* 278, E992–E999. doi:10.1152/ajpendo.2000.278.6.E992

Padilha, C. S., Borges, F. H., Costa Mendes da Silva, L. E., Frajacomo, F. T. T., Jordao, A. A., Duarte, J. A., et al. (2017). Resistance exercise attenuates skeletal muscle oxidative stress, systemic pro-inflammatory state, and cachexia in Walker-256 tumor-bearing rats. *Appl. Physiology, Nutr. Metabolism* 42, 916–923. doi:10.1139/apnm-2016-0436

Padilha, C. S., Cella, P. S., Chimin, P., Voltarelli, F. A., Marinello, P. C., De Jesus Testa, M. T., et al. (2021). Resistance training's ability to prevent cancer-induced muscle atrophy extends anabolic stimulus. *Med. Sci. Sports Exerc* 53, 1572–1582. doi:10.1249/MSS.0000000000002624

Padilha, C. S., Cella, P. S., Ribeiro, A. S., Voltarelli, F. A., Testa, M. T. J., Marinello, P. C., et al. (2019). Moderate vs high-load resistance training on muscular adaptations in rats. *Life Sci.* 238, 116964. doi:10.1016/j.lfs.2019.116964

Pearen, M. A., Ryall, J. G., Lynch, G. S., and Muscat, G. E. (2009). Expression profiling of skeletal muscle following acute and chronic beta2-adrenergic stimulation: implications for hypertrophy, metabolism and circadian rhythm. *BMC Genomics* 10, 448. doi:10.1186/1471-2164-10-448

Pedersen, B. K. (2013). Muscle as a secretory organ. *Compr. Physiol.* 3, 1337–1362. doi:10.1002/cphy.c120033

Phillips, S. M., Tipton, K. D., Aarsland, A., Wolf, S. E., and Wolfe, R. R. (1997). Mixed muscle protein synthesis and breakdown after resistance exercise in humans. *Am. J. Physiology-Endocrinology Metabolism* 273, E99–E107. doi:10.1152/ajpendo.1997.273.1.E99

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

Pillon, N. J., Gabriel, B. M., Dollet, L., Smith, J. A. B., Sardón Puig, L., Botella, J., et al. (2020). Transcriptomic profiling of skeletal muscle adaptations to exercise and inactivity. *Nat. Commun.* 11, 470. doi:10.1038/s41467-019-13869-w

Raastad, T., Bjøro, T., and Hallén, J. (2000). Hormonal responses to high- and moderate-intensity strength exercise. *Eur. J. Appl. Physiol.* 82, 121–128. doi:10.1007/s004210050661

Risling, T. E., Caulkett, N. A., and Florence, D. (2012). Open-drop anesthesia for small laboratory animals. *Can. veterinary J. = La revue veterinaire Can.* 53, 299–302.

Rodrigues, A. C. Z., Messi, M. L., Wang, Z., Abba, M. C., Pereyra, A., Birbrair, A., et al. (2019). The sympathetic nervous system regulates skeletal muscle motor innervation and acetylcholine receptor stability. *Acta Physiol.* 225, e13195. doi:10.1111/apha.13195

Rommel, C., Bodine, S. C., Clarke, B. A., Rossman, R., Nunez, L., Stitt, T. N., et al. (2001). Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. *Nat. Cell Biol.* 3, 1009–1013. doi:10.1038/ncb1101-1009

Rose, A. J., Kiens, B., and Richter, E. A. (2006). Ca $^{2+}$ -calmodulin-dependent protein kinase expression and signalling in skeletal muscle during exercise. *J. Physiol.* 574, 889–903. doi:10.1113/jphysiol.2006.111757

Rowell, L. B., and O'Leary, D. S. (1990). Reflex control of the circulation during exercise: chemoreflexes and mechanoreflexes. *J. Appl. Physiol.* 69, 407–418. doi:10.1152/jappl.1990.69.2.407

Ruas, J. L., White, J. P., Rao, R. R., Kleiner, S., Brannan, K. T., Harrison, B. C., et al. (2012). A PGC-1α isoform induced by resistance training regulates skeletal muscle hypertrophy. *Cell* 151, 1319–1331. doi:10.1016/j.cell.2012.10.050

Ryall, J. G., Schertzer, J. D., and Lynch, G. S. (2007). Attenuation of age-related muscle wasting and weakness in rats after formoterol treatment: therapeutic implications for sarcopenia. *journals gerontology. Ser. A, Biol. Sci. Med. Sci.* 62, 813–823. doi:10.1093/gerona/62.8.813

Santana, F. M., Domiciano, D. S., Gonçalves, M. A., Machado, L. G., Figueiredo, C. P., Lopes, J. B., et al. (2019). Association of appendicular lean mass, and subcutaneous and visceral adipose tissue with mortality in older Brazilians: the São Paulo ageing and health study. *J. Bone Mineral Res.* 34, 1264–1274. doi:10.1002/jbmr.3710

Sartori, R., Romanello, V., and Sandri, M. (2021). Mechanisms of muscle atrophy and hypertrophy: implications in health and disease. *Nat. Commun.* 12, 330–412. doi:10.1038/s41467-020-20123-1

Schiaffino, S., and Mammucari, C. (2011). Regulation of skeletal muscle growth by the IGF1-Akt/PKB pathway: insights from genetic models. *Skelet. Muscle* 1, 4. doi:10.1186/2044-5040-1-4

Schmittgen, T. D., and Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. *Nat. Protoc.* 3, 1101–1108. doi:10.1038/nprot.2008.73

Schnyder, S., and Handschin, C. (2015). Skeletal muscle as an endocrine organ: PGC-1α, myokines and exercise. *Bone* 80, 115–125. doi:10.1016/j.bone.2015.02.008

Shaywitz, A. J., and Greenberg, M. E. (1999). CREB: a stimulus-induced transcription factor activated by A diverse array of extracellular signals. *Annu. Rev. Biochem.* 68, 821–861. doi:10.1146/annurev.biochem.68.1.821

Silveira, W. A., Gonçalves, D. A., Graça, F. A., Andrade-Lopes, A. L., Bergantin, L. B., Zanon, N. M., et al. (2014). Activating cAMP/PKA signaling in skeletal muscle suppresses the ubiquitin-proteasome-dependent proteolysis: implications for sympathetic regulation. *J. Appl. Physiol.* 117, 11–19. doi:10.1152/japplphysiol.01055.2013

Silveira, W. A., Gonçalves, D. A., Machado, J., Lautherbach, N., Lustrino, D., Paula-Gomes, S., et al. (2020). cAMP-dependent protein kinase inhibits FoxO activity and regulates skeletal muscle plasticity in mice. *FASEB J.* 34, 12946–12962. doi:10.1096/fj.201902102RR

Silvestre, J. G. O., Speretta, G. F. F., Fabrizzi, F., Moraes, G., and Duarte, A. C. G. de O. (2017). Acute effects of Resistance exercise performed on ladder on energy metabolism, stress, and muscle damage in rats. *Mot. Rev. Educ. Física* 23. doi:10.1590/s1980-6574201700si0010

Takemori, H., Katoh Hashimoto, Y., Nakae, J., Olson, E. N., and Okamoto, M. (2009). Inactivation of HDAC5 by SIK1 in AICAR-treated C2C12 myoblasts. *Endocr. J.* 56, 121–130. doi:10.1507/endocrj.K08E-173

Testa, M. T. de J., Cella, P. S., Marinello, P. C., Frajacomo, F. T. T., Padilha, C. de S., Perandini, P. C., et al. (2022). Resistance training attenuates activation of STAT3 and muscle atrophy in tumor-bearing mice. *Front. Oncol.* 12, 880787. doi:10.3389/fonc.2022.880787

Thomson, D. M., Herway, S. T., Fillmore, N., Kim, H., Brown, J. D., Barrow, J. R., et al. (2008). AMP-activated protein kinase phosphorylates transcription factors of the CREB family. *J. Appl. Physiol.* 104, 429–438. doi:10.1152/japplphysiol.00900.2007

Wilkinson, S. B., Phillips, S. M., Atherton, P. J., Patel, R., Yarasheski, K. E., Tarnopolsky, M. A., et al. (2008). Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *J. Physiol.* 586, 3701–3717. doi:10.1113/jphysiol.2008.153916

Wojtaszewski, J. F. P., Nielsen, P., Hansen, B. F., Richter, E. A., and Kiens, B. (2000). Isoform-specific and exercise intensity-dependent activation of 5′-AMP-activated protein kinase in human skeletal muscle. *J. Physiol.* 528, 221–226. doi:10.1111/j.1469-7793.2000.t01-1-00221.x

Xiao, R. P. (2001). Beta-adrenergic signaling in the heart: dual coupling of the beta2-adrenergic receptor to G(s) and G(i) proteins. *Science's STKE signal Transduct. Knowl. Environ.* 2001, re15. doi:10.1126/stke.2001.104.re15

Yang, J., Zong, C. S., Xia, W., Yamaguchi, H., Ding, Q., Xie, X., et al. (2008). ERK promotes tumorigenesis by inhibiting FOXO3a via MDM2-mediated degradation. *Nat. Cell Biol.* 10, 138–148. doi:10.1038/ncb1676

Yimlamai, T., Dodd, S. L., Borst, S. E., and Park, S. (2005). Clenbuterol induces muscle-specific attenuation of atrophy through effects on the ubiquitin-proteasome pathway. *J. Appl. physiology (Bethesda, Md. 1985)* 99, 71–80. doi:10.1152/japplphysiol.00448.2004

Zhou, H.-H., Liao, Y., Peng, Z., Liu, F., Wang, Q., and Yang, W. (2023). Association of muscle wasting with mortality risk among adults: a systematic review and meta-analysis of prospective studies. *J. cachexia, sarcopenia muscle* 14, 1596–1612. doi:10.1002/jcsm.13263

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 09 February 2024
DOI 10.3389/fphys.2024.1357172

OPEN ACCESS

EDITED BY
Cristina Vassalle,
Gabriele Monasterio Tuscany Foundation (CNR), Italy

REVIEWED BY
Luca Petrigna,
University of Catania, Italy
Alessandro Pingitore,
National Research Council, Italy

*CORRESPONDENCE
Lina Majed,
limajed@hbku.edu.qa

RECEIVED 17 December 2023
ACCEPTED 30 January 2024
PUBLISHED 09 February 2024

CITATION
Majed L, Ibrahim R, Lock MJ and Jabbour G (2024), Walking around the preferred speed: examination of metabolic, perceptual, spatiotemporal and stability parameters. *Front. Physiol.* 15:1357172. doi: 10.3389/fphys.2024.1357172

Walking around the preferred speed: examination of metabolic, perceptual, spatiotemporal and stability parameters

Lina Majed[1]*, Rony Ibrahim[2], Merilyn Jean Lock[1] and Georges Jabbour[2]

[1]Exercise Science, Health and Epidemiology Division, College of Health and Life Sciences, Hamad Bin Khalifa University, Doha, Qatar, [2]Physical Education Department, College of Education, Qatar University, Doha, Qatar

Walking is the most accessible and common type of physical activity. Exercising at one's self-selected intensity could provide long-term benefits as compared to following prescribed intensities. The aim of this study was to simultaneously examine metabolic, perceptual, spatiotemporal and stability parameters at an absolute 3 km·h^{-1} speed range around the individual preferred walking speed (PWS). Thirty-four young sedentary adults (18 women) volunteered to walk at seven speeds relative to their PWS in 3-min trials interspaced with 3-min rest intervals. Results indicated a significant main effect of speed on all studied variables. While metabolic, perceptual and spatiotemporal values were sensitive to the smallest change in speed (i.e., 0.5 km·h^{-1}), a significant increase in the rate of carbohydrate oxidation and decrease in %fat oxidation were only observed at speeds above PWS. Results also revealed significantly higher coefficients of variation for stride characteristics at speeds below PWS only. Moreover, analyses of best fit models showed a quadratic relationship between most variables and speed, with the exceptions of metabolic cost of transport, rating of perceived exertion and stride duration that changed exponentially with speed. PWS coincided with optimized mechanical efficiency, fuel oxidation and gait stability. This indicated that walking below PWS decreased both mechanical efficiency and stability of gait, while walking above PWS increased carbohydrate oxidation. Those factors seem to play an important role as determinants of PWS. We suggest that walking at PWS may provide benefits in terms of fat oxidation while optimizing gait stability.

KEYWORDS

gait, perceived exertion, self-selected intensity, stability, fuel oxidation, energy expenditure, stride parameters, exercise

1 Introduction

Walking is the most natural, accessible, and common daily lifestyle activity. Although walking is viewed as a simple skill, it is considered as a complex behavior resulting from a large number of components (e.g., nervous and sensory systems, muscles, bones, joints) interacting to produce a stable pattern (Diedrich and Warren, 1995). While most people can walk at speeds reaching up to about 9 km·h^{-1}, they naturally choose to walk at a typical speed (i.e., around 4.5 km·h^{-1}) known as the preferred walking speed (PWS) (Bohannon and Andrews, 2011). From a dynamic perspective, walking at PWS is a behavior known to

possess properties of an attractor, in which any small change in the speed (i.e., control parameter) in either direction would result in loss of stability (Diedrich and Warren, 1995). According to self-optimization theories (Sparrow and Newell, 1998; Alexander, 2002), Humans naturally tend to adopt behaviors that minimize metabolic energy cost and other cost functions (e.g., mechanical, perceptual, cognitive). The relationship between walking speed and metabolic cost of transport has long been described as a U-shaped curve (Ralston, 1958; Zarrugh, Todd and Ralston, 1974; Margaria, 1976), indicating an optimum at PWS. Several studies have attempted to identify determinants of preferred behaviors or preferred intensities. Indeed, walking at PWS has been recognized to optimize not only energy cost, but also substrate utilization (Willis et al., 2005), biomechanical parameters (Saibene and Minetti, 2003), perceived exertion (Willis et al., 2005; Godsiff et al., 2018), cognitive function (Abernethy et al., 2002), and gait stability (Jordan et al., 2007). For instance, Willis et al. (2005) concluded that healthy young adults choose a PWS that minimizes carbohydrate oxidation rate. While there seems to be several competing factors being optimized at PWS (Fernández Menéndez et al., 2019), determinants of the spontaneous adoption of PWS are still a topic of debate.

From an evolutionary perspective, people are instinctively inclined to optimize their physical exertion when there is no need obtain food (Eaton and Eaton, 2003). In today's modern societies, meeting minimal physical activity recommendations for health requires a cognitive effort (Peters et al., 2002), and a certain level of motivation that becomes even more challenging when exercise is recommended or performed at higher intensities (Perri et al., 2002; Parfitt and Hughes, 2009; Ekkekakis et al., 2011). From a behavioral perspective, exercising at one's self-selected or preferred intensity may contribute to greater health benefits in the long-term (Ekkekakis et al., 2008; Williams, 2008). Walking is a major activity recommendation for health and disease prevention (Carnethon et al., 2009; Williams et al., 2014), therefore more focus is needed to better understand the multiple acute responses when walking at or around the preferred intensity as a primary mode of exercise, especially in sedentary individuals.

Specific physical activity guidelines or exercise recommendations usually relate to frequency, duration, and intensity (e.g., 40%–59% $\dot{V}O_2$ reserve or RPE of 12–13) that might not be as accessible or as effective for changing behavior in inexperienced populations compared to just "moving" the way they prefer. For example, previous experimental studies have found that low-active, overweight adults volitionally completed more minutes per week of self-paced walking compared to walking regulated using prescribed heart rates (Williams et al., 2015).

Walking parameters have been analyzed at and around PWS, and studies have investigated how changes in walking speed affect gait and other acute responses. However, most studies have used a range of set absolute speeds for all participants (Willis et al., 2005; Fernández Menéndez et al., 2019), or expressed speed as a percentage of PWS (Jordan et al., 2007; Chung and Wang, 2010), or even defined speed as slow, self-selected and fast (Haight et al., 2014). The present study aims to examine metabolic, perceptual, spatiotemporal and gait stability parameters at an absolute range of speeds (3 km·h^{-1}) around each participant's own PWS. We hypothesized that sedentary young adults choose to walk at speeds that optimize certain cost function (e.g., metabolic cost, perceived exertion, stability), while providing benefits in terms of fuel oxidation (Willis et al., 2005). Evidence of optimization at PWS is expected to be seen following one of two criteria. First, the minimum or maximum of a quadratic relationship between the studied variable and speed should coincide with PWS indicating an optimum. Second, with increasing speeds evidence of a deflection in values of the studied variable should happen at PWS. In addition to examining determinants of PWS, the present study further attempts to understand potential benefits of walking at or around the PWS, rather than at intensities relative to the maximum or reserve heart rate or oxygen consumption.

TABLE 1 Participants' physical characteristics and preferred walking speeds (PWS). Data are presented as mean (standard deviation).

	Female	Male	All
Sample size	18	16	34
Age (years)	21.67 (1.91)	23.49 (2.28)*	22.52 (2.26)
Body height (cm)	158.06 (4.57)	174.31 (7.15)**	165.71 (10.09)
Body mass (kg)	57.42 (14.16)	72.89 (18.76)*	64.70 (18.02)
Body mass index	22.90 (5.08)	23.81 (5.04)	22.52 (2.26)
Preferred walking speed (m·s^{-1})	1.12 (0.12)	1.16 (0.19)	1.14 (0.16)
Preferred walking speed (km·h^{-1})	4.03 (0.43)	4.18 (0.68)	4.10 (0.58)

*$p < 0.05$, **$p < 0.01$.

2 Materials and methods

2.1 Participants

Thirty-four healthy volunteers (18 women and 16 men) aged between 18 and 26 years old took part in the study that was approved by the local Ethics Committee (QU-IRB 1696-E/22). Participants were recruited from the student body via word of mouth and through advertisement (i.e., flyers) shared at various locations of the university campus. Participants were sedentary (i.e., low physical activity level), non-smokers and did not present any past or present disease (e.g., neurological, cardiovascular, respiratory, metabolic diseases), or other health complications (e.g., injury, motor impairment) that might interfere with their ability to walk normally. None of them was taking any medication and their body mass did not vary more than 2.5 kg in the last 3 months. Participants' physical characteristics are shown in Table 1. Prior to the experiment, a written informed consent was obtained from each volunteer in accordance with the Declaration of Helsinki.

2.2 Protocol

Participants reported once to the laboratory after a 10–12 h overnight fast, wearing comfortable sports outfits and footwear. They were instructed to refrain from exercise and caffeinated products in the 24 h preceding the experiment. All tests were

conducted in the morning (between 9a.m. and 12p.m.) and under similar environmental conditions (i.e., 55% relative humidity and 22°C).

After an initial physical activity screening using the short-form of the International Physical Activity Questionnaire (IPAQ-SF, Craig et al., 2003), body mass and height were collected (i.e., body mass and body height). A 10-min familiarization with treadmill was carried out (Meyer et al., 2019) to ensure that participants' gait was representative of treadmill walking and a stable performance was reached. The protocol was then divided into two consecutive testing phases lasting approximately 2 h.

2.2.1 Determination of the preferred walking speed

A standardized treadmill test was used to determine the individual preferred walking speed (PWS) (Jordan et al., 2007; Dal et al., 2010). Participants, blind to the displayed digital speed, started by walking at a slow pace of 2 km·h^{-1}, after which increments of 0.1 km·h^{-1} followed every 10 s until the most comfortable speed was reported. At that stage, the treadmill's speed increased by 1.5 km·h^{-1} and speed decrements of 0.1 km·h^{-1} followed every 10 s until participants reported once again reaching their PWS. This procedure was repeated three times with a 3-min rest interval between trials. The PWS was then calculated as the mean of the six reported speed values.

2.2.2 Walking test

The second experimental phase was performed after a 10-min seated rest and aimed to collect physiological, perceptual, and spatiotemporal parameters for walking at different absolute speed levels around the PWS. The test consisted in seven 3-min walking trials at speeds relative to the PWS (i.e., in the following order: PWS-1.5 km·h^{-1}, PWS-1 km·h^{-1}, PWS-0.5 km·h^{-1}, PWS, PWS+0.5 km·h^{-1}, PWS+1 km·h^{-1} and PWS+1.5 km·h^{-1}). Speed trials were interspaced by a 3-min resting period to allow enough recovery and avoid any fatigue effect. Prior to testing, participants were instructed on how to report their rating of perceived exertion (RPE) by raising their index finger to indicate their score while the experimenter read the scale up from 6 to 20 (Borg, 1973). Finally, participants were fitted with the gas analyzer and heart rate monitor.

2.3 Apparatus

All walking trials were performed on a motorized treadmill (Valiant 2 CPET, Lode, the Netherlands) set at a gradient of 0%. A metabolic gas analyzer was used to collect respiratory and gas exchange data (Metalyzer 3B with MetaSoft Studio software, Cortex Medical, Germany). A standardized calibration procedure was undertaken before each test according to the manufacturer's instructions for ambient air, reference gases of known concentrations (with a gas bottle) and airflow volume (with a 3-L syringe) (Medbo et al., 2002). A synchronized heart rate polar chest belt (Polar, Kempele, Finland) and a 6–20 Borg scale (Borg, 1973) were used for heart rate data and ratings of perceived exertion (i.e., 6 for no exertion at all, 20 for maximal exertion).

A high-definition camcorder (CCD-TRV66, Sony, Japan) recorded the intermediate 1-min interval of each of the seven 3-min walking trials at a frequency of 25 Hz. The camera was set at a standard position perpendicular to the left mid-point of the treadmill's long axis at a 2-m distance. Participants were equipped with two reflective markers on their left heel (i.e., calcaneus) and toe (i.e., second metatarsal head) to allow the detection of heel-strike and toe-off events. A video-based analysis and modeling software were used to digitalize and compute spatiotemporal data (Tracker 4.91, Open Source Physics; Brown, 2015).

2.4 Data processing

2.4.1 Physiological and perceptual data

Oxygen consumption ($\dot{V}O_2$, L·min^{-1}), carbon dioxide production ($\dot{V}CO_2$, L·min^{-1}), minute ventilation ($\dot{V}$ E, L·min^{-1}) and heart rate (HR, beats·min^{-1}) were continuously recorded during the seven walking trials. Respiratory exchange ratio (RER) was computed as the ratio between $\dot{V}CO_2$ and $\dot{V}O_2$. Mean values were determined at the last minute of each trial when a steady state was reached. The relative oxygen consumption (r $\dot{V}O_2$, mL·kg^{-1}·min^{-1}) was calculated from the individual body mass values. The net relative $\dot{V}O_2$ per distance traveled was computed to obtain the metabolic cost of transport (MCT, mL·kg^{-1}·km^{-1}, di Prampero, 1986) with speed expressed in km·h^{-1} and a resting $\dot{V}O_2$ value set at 3.5 mL·kg^{-1}·min^{-1} (Medbo and Tabata, 1989). Steady state $\dot{V}O_2$ and $\dot{V}CO_2$ values were used to compute the energy expenditure (EE, kcal·min^{-1}) according to Brouwer (1957). Percent fat oxidation (%Fat) was calculated in relation to the mean steady state non-protein RER following McGilvery and Goldstein's (1983) method. The rates of fat and carbohydrate (CHO) oxidation were calculated in g. min^{-1} according to the non-protein RER (Peronnet and Massicotte, 1991). The values were then expressed in gA78Fkg^{-1}·h^{-1} relatively to body mass.

Gross mechanical efficiency (ME) was computed based on Lafortuna et al.'s (2006) equation, as the ratio of work expressed as the absolute speed in km·h^{-1} and the rate of energy consumed (E, W. min^{-1}) that was in turn calculated according to Garby and Astrup (1987). RPE values were collected exactly 20 s before the end of each 3-min walking trial when participants raised the index finger to indicate their RPE value as the experimenter read up the scale.

2.4.2 Movement-related data

Spatiotemporal gait parameters were obtained by examining 15 full strides recorded in the middle of each trial (i.e., between the first and second minutes) (Hansen et al., 2017). For each stride, the exact timings (i.e., frames) at which the left heel touched the ground (i.e., heel strike) and the left toe left the ground (i.e., toe-off) were recorded by an experienced researcher. [1] Stride duration (SD, s) represented the time between two consecutive left heel strikes, [2] support phase duration (SPD, s) was considered as the time elapsed between left heel strike and the consecutive left toe off within the same stride, [3] stride length (SL, m·stride^{-1}) was computed as the product of the SD and the corresponding trial's speed (in m·s^{-1}) and [4] stride frequency (SF, stride·s^{-1}) was calculated as the ratio between the corresponding speed (in m·s^{-1}) and SL. Furthermore, the coefficient of variation (CoV) for SD, SPD, SF and SL were calculated as the ratio between the standard deviation and the mean of values relative to the 15 individual strides collected at each speed

10.3389/fphys.2024.1357172

TABLE 2 ANOVAs' results for the effect of speed and gender on physiological and perceptual variables.

	Tested effect	F (df_1,df_2)	η^2	*p*
r $\dot{V}O_2$ (mL·kg^{-1}·min^{-1})	Speed	F (2.110,67.515) = 282.626	0.898	<0.001
	Gender	F (1.32) = 2.386	0.069	0.132
	Speed × Gender	F (2.110,67.515) = 1.766	0.052	0.177
EE (kcal·min^{-1})	Speed	F (1.788,57.212) = 205.622	0.865	<0.001
	Gender	F (1.32) = 15.425	0.325	<0.001
	Speed × Gender	F (1.788,57.212) = 0.683	0.021	0.493
MCT (mL·kg^{-1}·km^{-1})	Speed	F (2.959,94.701) = 407.584	0.927	<0.001
	Gender	F (1.32) = 2.023	0.059	0.165
	Speed × Gender	F (2.959,94.701) = 3.054	0.087	0.033
ME (%)	Speed	F (2.076,66.429) = 61.959	0.659	<0.001
	Gender	F (1.32) = 14.841	0.317	<0.001
	Speed × Gender	F (2.076,66.429) = 0.289	0.009	0.758
HR (beats·min^{-1})	Speed	F (2.061,65.948) = 175.487	0.846	<0.001
	Gender	F (1.32) = 4.661	0.127	0.038
	Speed × Gender	F (2.061,65.948) = 3.208	0.091	0.045
$\dot{V}_E$ (L·min^{-1})	Speed	F (2.656,85.003) = 116.322	0.784	<0.001
	Gender	F (1.32) = 17.507	0.354	<0.001
	Speed × Gender	F (2.656,85.003) = 0.088	0.003	0.954
%Fat (%)	Speed	F (4.150,132.784) = 11.683	0.267	<0.001
	Gender	F (1.32) = 0.337	0.010	0.566
	Speed × Gender	F (4.150,132.784) = 0.924	0.028	0.455
Fat (g·kg^{-1}·h^{-1})	Speed	F (3.945,126.253) = 2.876	0.082	0.026
	Gender	F (1.32) = 0.008	0.000	0.929
	Speed × Gender	F (3.945,126.253) = 1.186	0.036	0.320
CHO (g·kg^{-1}·h^{-1})	Speed	F (3.018,96.561) = 42.697	0.572	<0.001
	Gender	F (1.32) = 0.861	0.026	0.360
	Speed × Gender	F (3.018,96.561) = 0.714	0.022	0.546
RPE	Speed	F (1.925,61.585) = 55.077	0.633	<0.001
	Gender	F (1.32) = 1.249	0.038	0.272
	Speed × Gender	F (1.925,61.585) = 1.211	0.036	0.304

r $\dot{V}O_2$, relative oxygen consumption; EE, energy expenditure; MCT, metabolic cost of transport; ME, mechanical efficiency; HR, heart rate; $\dot{V}_E$, minute ventilation; %Fat, percent fat oxidation; Fat/CHO, rate of fat/carbohydrate oxidation relative to body mass; RPE, rating of perceived exertion.

trial. The latter was meant to account for dynamic stability in gait as done by Fischer et al. (2021). Speed is presented in km·h^{-1} in the text for comparison purposes.

2.5 Statistical analysis

The normality of all data sets was verified using Shapiro-Wilk's tests and by visual inspection of Q-Q plots. Independent *t*-tests were used to compare physical characteristics and PWS between male and female groups. Mixed analyses of variances (ANOVAs) were performed to examine main and interaction effects of Speed (7 repeated measures in km·h^{-1}: PWS-1.5, PWS-1, PWS-0.5, PWS, PWS+0.5, PWS+1 and PWS+1.5) and Gender (between-subject factor) on all physiological (i.e., r $\dot{V}O_2$, EE, MCT, ME, HR, $\dot{V}$ E, %Fat, Fat, CHO), perceptual (i.e., RPE) and movement-related (i.e., SF, SL, SD, SPD, and their CoVs) variables. Assumptions of homogeneity and sphericity were verified and a Huynh-Feldt procedure was used to adjust the significance of *p*-values and F (degrees of freedom) to control for possible

10.3389/fphys.2024.1357172

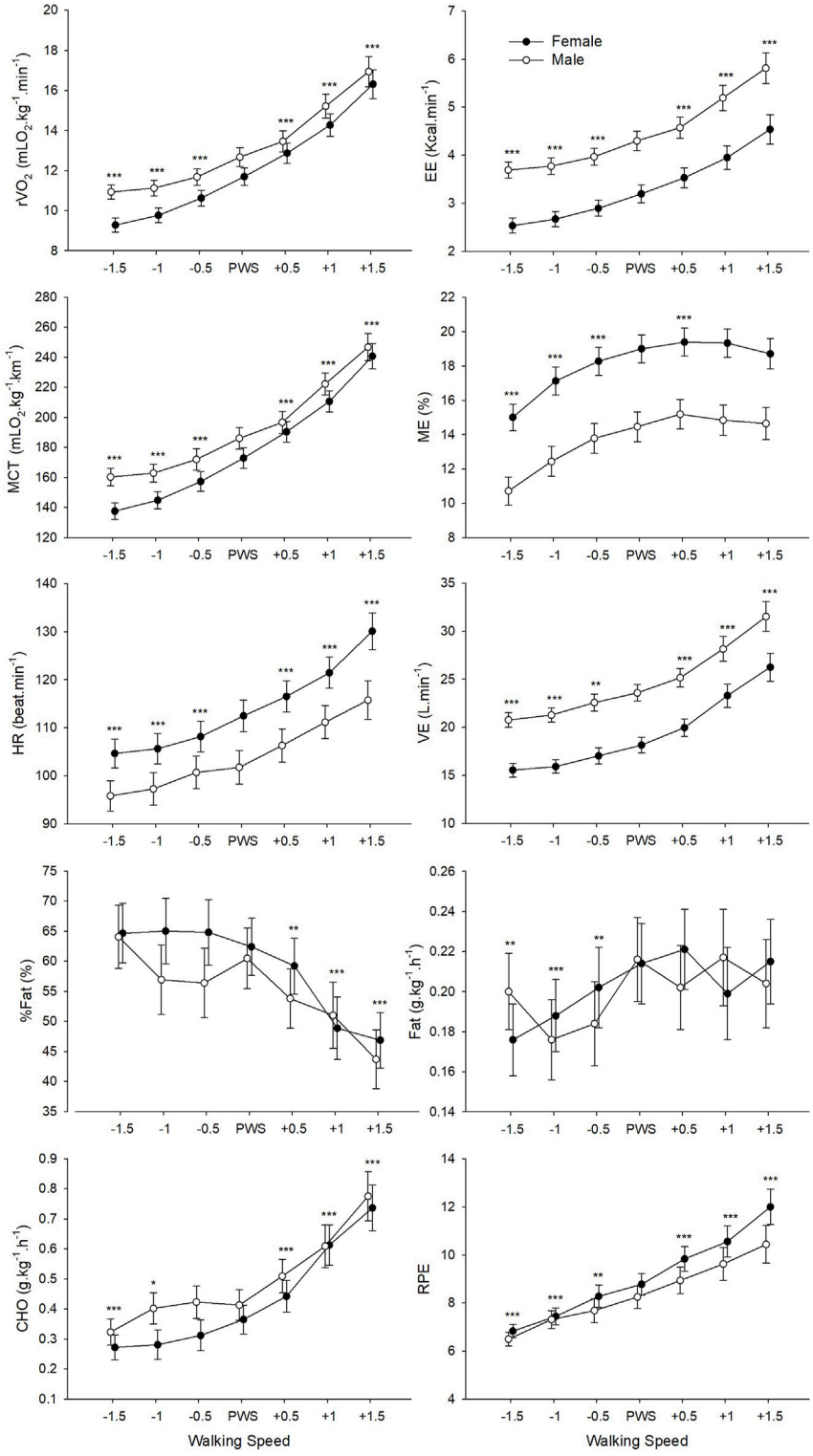


FIGURE 1
Mean values of physiological and perceptual variables for male and female groups according to the seven tested speeds relative to the preferred walking speed (PWS) in km·h^{-1}. r $\dot{V}O_2$, relative oxygen consumption; EE, energy expenditure; MCT, metabolic cost of transport; ME, mechanical efficiency; HR, heart rate; $\dot{V}$ E, minute ventilation, %Fat, percent fat oxidation; Fat/CHO, rate of fat/carbohydrate oxidation relative to body weight; RPE, rating of perceived exertion. Error bars represent the standard error. Significant speed-related differences are presented in comparison to PWS, *p < 0.05, **p < 0.01, ***p < 0.001.

violations (Huynh and Feldt, 1970). Analyses were completed when needed with pairwise *post hoc* comparisons with Bonferroni adjustments to the significance level. Best fit regression models were performed to examine the relationship between each dependent variable and relative walking speeds. The best fit model was considered only if the regression model was significant (ANOVA results, $p < 0.05$) and presented the highest adjusted coefficient of determination (R^2) value compared to other models. When the adjusted R^2 values were similar, the model with the highest F value was retained. All data are presented as mean and standard deviation. Statistical analyses were performed using SPSS (IBM, version 28) with a level of significance set at $p < 0.05$.

3 Results

3.1 Physical characteristics

PWS of the male and female groups did not differ significantly ($p > 0.050$, Table 1). Male participants were on average 1.82 years older than female participants [t (32) = 2.535, $p = 0.016$], while no significant differences in their body mass index (BMI) was present (Table 1). Body mass and body height were significantly higher for the male group as compared to the female group [t (32) = 2.732, $p = 0.010$; t (32) = 7.989, $p < 0.001$, respectively].

3.2 Physiological and perceptual data

ANOVAs revealed a significant main effect of Speed on all physiological data and RPE (Table 2; Figure 1). Post-hoc pairwise comparisons made relative to PWS are displayed in detail in Figure 1. In general, values of most variables (i.e., r $\dot{V}O_2$, EE, MCT, HR, $\dot{V}$ E, RPE) changed significantly at every speed increment and presented significant differences between all speeds. However, the significant increase in ME values with speeds was only seen between PWS-1.5 and PWS+0.5 km·h^{-1} after which no further increases were noted. Moreover, %Fat values did not change with speed until PWS+0.5 km·h^{-1} where a significant decline was revealed with increasing speeds (Figure 1). Similarly, the rate of Fat oxidation increased significantly with speed until PWS then stabilized, while the opposite was found for the rate of CHO oxidation that increased significantly only at speeds higher than PWS. ANOVAs also revealed a significant main effect of gender on EE, ME, HR and $\dot{V}$ E (Table 2). Although male participants had significantly higher EE and $\dot{V}$ E values as compared to female participants, they presented significantly lower ME and HR (Figure 1). Significant interaction effects were also noted for MCT and HR, indicating no significant gender differences for MCT at speeds higher than PWS-1 km·h^{-1} and for HR at speeds lower than PWS ($p > 0.05$).

3.3 Movement-related data

ANOVAs found a significant main effect of Speed on all studied movement-related variables (Table 3). For SF, SL, SD, and SPD, a significant change in values was observed at each speed increment (Figure 2). CoV values for SF, SL, and SD presented a similar pattern of change with speed, where values were significantly higher at the lowest tested speed as compared to the four highest speeds (including PWS). The CoV for SPD presented the lowest values at PWS-0.5 km·h^{-1} and PWS as compared to lower (i.e., PWS-1.5 km·h^{-1}) and higher (i.e., PWS+1 km·h^{-1}) speeds. While no Gender effect appeared in the analyses, an interaction was found on SD and SPD indicating convergence of male and female values at higher speed values.

3.4 Regressions: best fit model

The relationship between variables and relative walking speed (i.e., PWS-1.5 to +1.5 km·h^{-1}) was further investigated. This analysis was done by examining the overall best fit regression model for each variable with data from female and male groups pooled together. Results showed that the relationship between most of the studied variables and speed was best described as quadratic (Table 4), with the exceptions of MCT, RPE, SD and SPD, all of which were best described with an exponential model. The rate of fat oxidation was not fitted significantly to any of the studied models (i.e., linear, quadratic, exponential). Adjusted coefficients of determination (R^2) varied from 3.1% to 69.4%, revealing a higher predictive power of speed for spatiotemporal variables as well as relative oxygen consumption and MCT. Figure 3 offers a better visualization of the actual best fit models for each studied variable. The optimum of quadratic functions is indicated by a dashed vertical line to point to the calculated vertex x-coordinate (i.e., speed).

4 Discussion

The present study investigated metabolic, perceptual, spatiotemporal and stability parameters when walking at a 3 km·h^{-1} range around the individual preferred walking speed (PWS). Main findings indicate that sedentary young adults do not select their PWS based on principles of minimization of metabolic cost (Ralston, 1958; Zarrugh, Todd and Ralston, 1974; Margaria, 1976) or based on perceived exertion (Willis et al., 2005; Fernández Menéndez et al., 2019). Participants preferred to walk at a speed that optimizes fuel oxidation (Willis et al., 2005), mechanical efficiency and gait stability, which confirms in part our hypothesis. Indeed, it seems that several competing factors are optimized at PWS (Fernández Menéndez et al., 2019). However, present results could not confirm the well-established U-shaped relationship between metabolic cost of transport and speed (Ralston, 1958; Zarrugh, Todd and Ralston, 1974; Margaria, 1976), which is in line with a more recent study (Willis et al., 2005). Our results support those demonstrating that PWS does not occur at a minimum energy cost (Godsiff et al., 2018). The absolute speed range considered in the current study (i.e., 0.5 km·h^{-1} increments from PWS-1.5 to PWS+1.5 km·h^{-1}) differed according to each participant's own PWS, which was not the case in previous studies examining the relationship between metabolic and perceptual responses to a set absolute speed continuum for all participants (Willis et al., 2005). Although this might make

10.3389/fphys.2024.1357172

TABLE 3 ANOVAs' results for the effect of speed and gender on movement related variables.

	Tested effect	F (df1,df2)	η^2	*p*
SF (stride·s^{-1})	Speed	F (2.127,68.071) = 653.731	0.953	<0.001
	Gender	F (1.32) = 1.345	0.040	0.255
	Speed × Gender	F (2.127,68.071) = 1.162	0.035	0.321
SL (m·stride^{-1})	Speed	F (2.656, 84.995) = 482.361	0.938	<0.001
	Gender	F (1.32) = 0.131	0.004	0.720
	Speed × Gender	F (2.656,84.995) = 0.619	0.019	0.585
SD (s)	Speed	F (1.747,55.892) = 239.431	0.882	<0.001
	Gender	F (1.32) = 1.615	0.048	0.213
	Speed × Gender	F (1.747,55.892) = 4.229	0.117	0.024
SPD (s)	Speed	F (3.005,96.175) = 215.900	0.871	<0.001
	Gender	F (1.32) = 0.220	0.007	0.642
	Speed × Gender	F (3.005,96.175) = 5.587	0.149	0.001
CoV SF	Speed	F (3.968,126.981) = 4.649	0.127	0.002
	Gender	F (1.32) = 2.578	0.075	0.118
	Speed × Gender	F (3.968,126.981) = 0.741	0.023	0.565
CoV SL	Speed	F (3.950,126.392) = 4.763	0.130	0.001
	Gender	F (1.32) = 2.557	0.074	0.120
	Speed × Gender	F (3.950,126.392) = 0.824	0.025	0.511
CoV SD	Speed	F (3.950, 126.392) = 4.763	0.130	0.001
	Gender	F (1.32) = 2.557	0.074	0.120
	Speed × Gender	F (3.950, 126.392) = 0.824	0.025	0.511
CoV SPD	Speed	F (5.055,161.775) = 4.447	0.122	<0.001
	Gender	F (1.32) = 0.146	0.005	0.705
	Speed × Gender	F (5.055,161.775) = 1.710	0.051	0.134

SD, stride duration; SPD, support phase duration; SF, stride frequency; SL, stride length; CoV, coefficient of variation.

comparisons harder, factors being optimized at PWS are more easily identified when individual PWS is considered.

The average PWS of the present sample was 4.1 (±0.58) km·h^{-1} which is in accordance with previous studies in Western (Bohannon and Andrews, 2011; Bohannon and Wang, 2019) and Arab populations (Majed et al., 2020; 2022). A first result indicated that walking speed significantly affected all studied metabolic, perceptual, spatiotemporal and stability parameters (Tables 2, 3). Participants were sensitive to the smallest change in speed (i.e., 0.5 km·h^{-1}) around PWS, which was also revealed by significant changes in the subjective perception of effort (i.e., RPE). Increasing speeds around PWS was linked to an increase in relative oxygen consumption, energy expenditure, metabolic cost of transport, heart rate, and RPE (Figure 1). Interestingly, certain parameters presented significant speed-related changes only below PWS or above PWS. For instance, values of mechanical efficiency and fat oxidation rate seem to stabilize close to PWS after initially increasing with speed. Moreover, a clear decline in %fat oxidation and a rise in carbohydrate oxidation rate are seen at speeds above PWS only, indicating a possible shift of substrate contribution to energy production (Willis et al., 2005). These important observations indicating a deflection point at or close to PWS in mechanical efficiency and substrate oxidation could be interpreted as potential determinants of PWS.

Findings indicate that participants choose to walk at a speed that not only optimizes mechanical efficiency, but also minimizes reliance on carbohydrate to potentially avoid fatigue (Willis et al., 2005). However, it remains unclear whether walking at PWS corresponds to the range of maximal rate of fat oxidation (Fat_{max}). Previous reports have indicated that the maximal rate of fat oxidation is expected to occur at low or moderate intensities around approximately 36%–65% $\dot{V}O_{2max}$ (Achten and Jeukendrup, 2004). In a recent study examining changes in the rate of fat oxidation during walking in sedentary young men (29.3 ± 0.7 years), authors reported maximal fat oxidation rates occurring at an average speed of 4.35 km·h^{-1} and corresponding to an intensity of 57.2% HR_{max} (Özdemir et al., 2019). Assuming an age-predicted

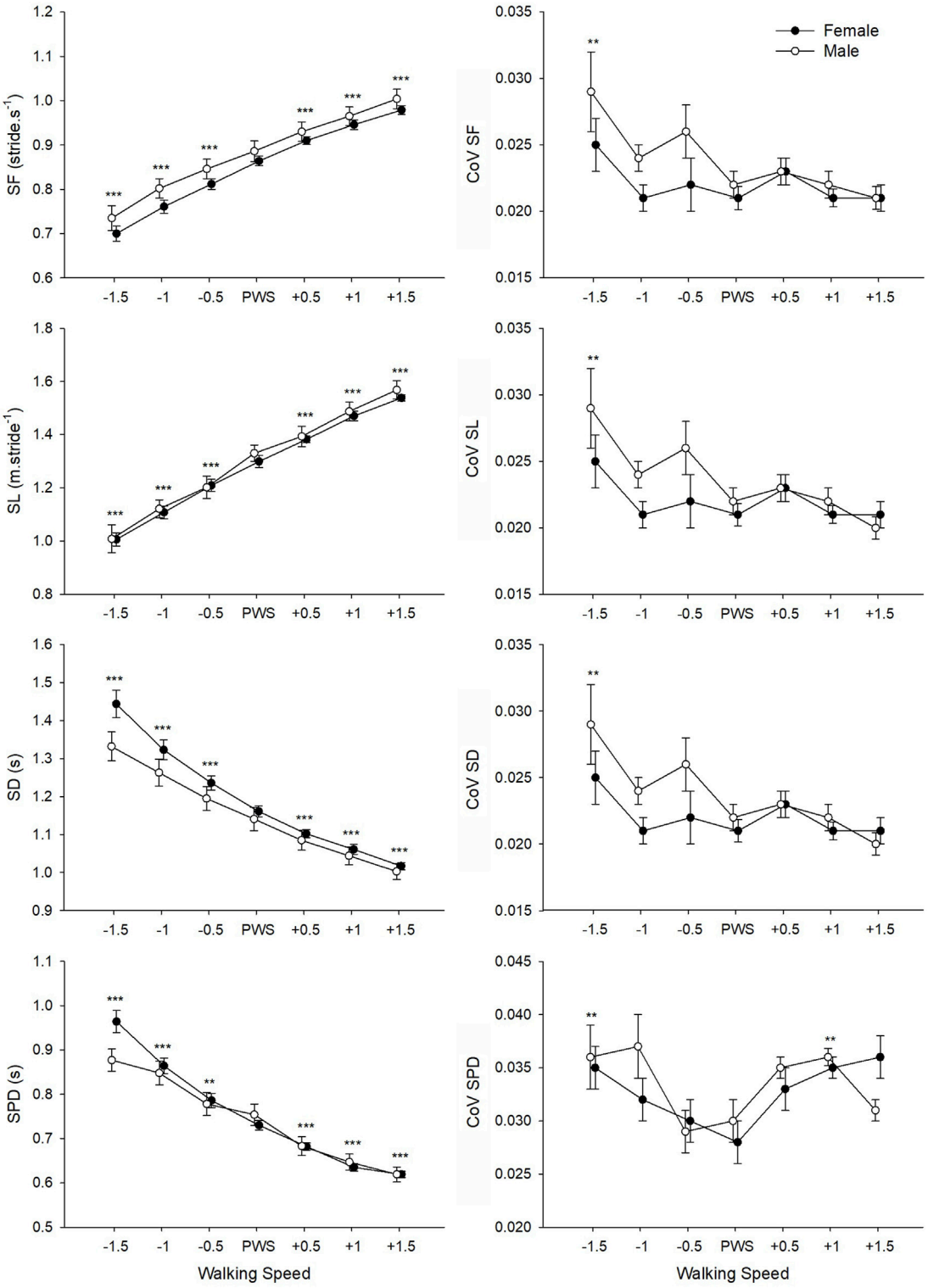


FIGURE 2
Mean values of movement-related variables for male and female groups according to the seven tested speeds relative to the preferred walking speed (PWS) in km·h^{-1}. SF, stride frequency; SL, stride length; SD, stride duration; SPD, support phase duration; CoV, coefficient of variation. Error bars represent the standard error. Significant speed-related differences are presented in comparison to PWS, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

maximal heart rate (i.e., 220–age) of 197.5 beats·min^{-1} for the present sample, it is possible that their PWS corresponded closely to predicted intensities (i.e., approximately 53% HR_{max}). This speculation cannot be confirmed from the present data given that no maximal testing was performed to accurately determine maximal capacity. Fat oxidation could be a desired process for weight loss or

10.3389/fphys.2024.1357172

TABLE 4 Best fit regression models for all tested variables in relation to relative walking speeds.

Variables	Best fit model	Adj R^2	SEE	F (2, 235)	Parameter estimates		
					Constant	b1	b2
r $\dot{V}$ O_2 (mL·kg^{-1}·min^{-1})	Quadratic	0.525	2.108	131.962***	12.035	2.165	0.566
EE (kcal·min^{-1})	Quadratic	0.311	1.043	53.089***	3.678	0.677	0.189
MCT (mL·kg^{-1}·km^{-1})	Exponential	0.592	1.140	344.444***	180.894	0.168	
ME (%)	Quadratic	0.109	4.064	15.515***	16.968	1.234	−0.917
HR (beats·min^{-1})	Quadratic	0.215	14.544	33.430***	107.487	7.527	1.922
$\dot{V}_E$ (L·min^{-1})	Quadratic	0.360	4.852	67.714***	20.747	3.516	1.176
%Fat (%)	Quadratic	0.076	20.994	10.803***	59.482	−5.984	−2.367
Fat (g·kg^{-1}·h^{-1})	ns	ns	ns	ns	ns	ns	
CHO (g·kg^{-1}·h^{-1})	Quadratic	0.293	0.236	50.086***	0.404	0.145	0.057
RPE	Exponential	0.322	0.231	113.330***	8.419	0.159	
SF (stride·s^{-1})	Quadratic	0.607	0.073	183.814***	0.876	0.090	−0.010
SL (m·stride^{-1})	Quadratic	0.694	0.121	270.322***	1.305	0.182	−0.011
SD (s)	Exponential	0.601	0.085	358.304***	1.162	−0.104	
SPD (s)	Exponential	0.654	0.099	448.427***	0.739	−0.136	
CoV SF	Quadratic	0.059	0.006	8.454***	0.022	−0.002	0.001
CoV SL	Quadratic	0.060	0.006	8.560***	0.022	−0.002	0.001
CoV SD	Quadratic	0.060	0.006	8.560***	0.022	−0.002	0.001
CoV SPD	Quadratic	0.031	0.008	4.843**	0.031	−0.000	0.002

Adj, adjusted; SEE, standard error of estimate; r $\dot{V}O_2$, relative oxygen consumption; EE, energy expenditure; MCT, metabolic cost of transport; ME, mechanical efficiency; HR, heart rate; $\dot{V}$ E, minute ventilation; %Fat, percent fat oxidation; Fat/CHO, rate of fat/carbohydrate oxidation relative to body weight; RPE, rating of perceived exertion; SF, stride frequency; SL, stride length; SD, stride duration; SPD, support phase duration; CoV, coefficient of variation. Significant model fit, $^{**}p < 0.01$, $^{***}p < 0.001$.

maintenance, and walking at PWS could present advantages in that sense. While protocols of 3-min stages have been proven to be sufficient for determining maximal fat oxidation rates (Achten and Jeukendrup, 2004), walking at PWS for longer periods reveals contradictory findings as to the sustainability of maximum fat oxidation state. For example, Özdemir et al. (2019) found that the maximal rate of fat oxidation during walking decreases in the first 16 min of exercise, however, earlier studies have demonstrated an increased contribution of fat to energy production with longer exercise durations (Klein et al., 1994; Ravussin et al., 1986). In an effort to make exercise recommendations for sedentary individuals, duration needs to be considered given that the present study only reports short-term acute responses to walking.

The examination of spatiotemporal and gait stability parameters offers further insights into determinants of PWS. In previous work, PWS was shown to correspond to a typical spatiotemporal organization of gait in which a specific combination of stride frequency (SF) and stride length (SL) is adopted (Saibene and Minetti, 2003). Additionally, SF seems to play an important role in selection of PWS, as the U-shaped curve found between SF and metabolic cost of transport indicates an optimum at the preferred SF in both adults and children (Holt, Hamill and Andres, 1991; Jeng, Liao, Lai and Hou, 1997). Optimal temporal characteristics related to stride frequency were successfully mathematically predicted based on the resonant period of a force-driven harmonic oscillator (Holt, Hamill and Andres, 1990). According to these authors, walking at the preferred SF requires less muscle forces to maintain gait and potentially less mechanical work by body segments. Our results do not show an optimum in SL or SF around PWS *per se*, as both parameters increased significantly at each speed increment. Nevertheless, it is important to note that the organization of spatiotemporal gait parameters presented significantly higher variability (i.e., coefficient of variation, CoV) at speeds below PWS. Variability in stride characteristics is considered an indirect measure of gait stability (Hamacher et al., 2011; Fischer et al., 2021). This indicates that walking slower than PWS was less stable, and stability increased with speed until PWS where no further changes were seen in most variables. Importantly, CoV of the support phase duration presented as the most appropriate factor in the search of a PWS determinant, as its quadratic relationship with speed indicates an optimum exactly at PWS (Figure 3). As noted by Jordan et al. (2007), walking at PWS presents an enhanced stability and reproducibility compared to walking slower or faster, and thus behaves like a stable attractor. Any change in walking speed from PWS would result in the loss of dynamic stability. The low CoV values found in this study (i.e., approximately 3%) correspond to those found in healthy adults (Beauchet et al., 2009; Fischer et al., 2021) and reflect the general stability of this well-learned and

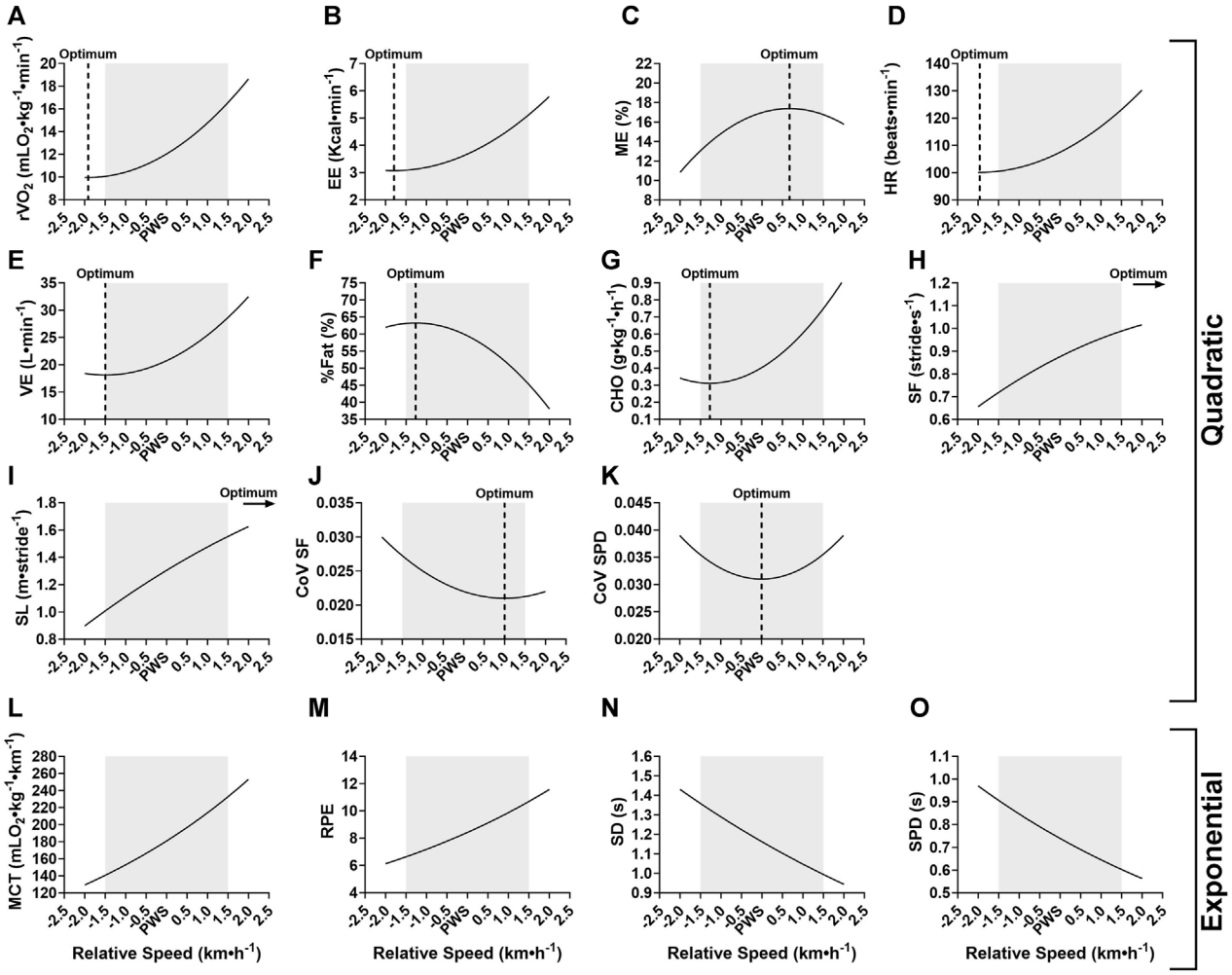


FIGURE 3
Visual representation of the best fit significant models for tested variables at speeds relative to the preferred walking speed (PWS) in km·h^{-1}. **(A)** r $\dot{V}O_2$, relative oxygen consumption; **(B)** EE, energy expenditure; **(C)** ME, mechanical efficiency; **(D)** HR, heart rate; **(E)** $\dot{V}$ E, minute ventilation, **(F)** %Fat, percent fat oxidation; **(G)** CHO, rate of carbohydrate oxidation relative to body weight; **(H)** SF: stride frequency, **(I)** SL, stride length; **(J)** CoV SF, coefficient of variation of the stride frequency (the same function applies to the CoV stride length and stride duration), **(K)** CoV SPD, coefficient of variation of the support phase duration, **(L)** MCT, metabolic cost of transport; **(M)** RPE, rating of perceived exertion; **(N)** SD, stride duration; **(O)** SPD, support phase duration. The light grey area represents the tested speed range. For quadratic models, the optimum (i.e., vertex) is represented with a vertical dashed line.

repetitive motor skill. Interestingly, when comparing factors influencing PWS, Fischer et al. (2021) reported significantly higher CoV of contact time (i.e., support phase duration) in patients with lung disease as compared to healthy control group. This brings further support to our finding on the importance of contact time in gait that is directly linked to stability and risk of falls (Fischer et al., 2021).

Gender differences seen in some physiological and metabolic variables (i.e., EE, ME, HR and $\dot{V}$ E) were not related to differences in speeds as both male and female groups had similar PWS values. It is safe to assume that anthropometric differences might have affected values that were not normalized to body mass (e.g., EE, $\dot{V}$ E). The gender-differences observed in heart rate could also be potentially due to a difference in stroke volume or aerobic capacity ($\dot{V}O_{2\ max}$), however this could not be verified in the present study.

In sum, Figure 3 offers a visual summary of findings with the best fit functions of all studied variables and indicates the calculated optimum for quadratic models. Most of the studied variables were best fitted with a quadratic model and the predicted optimum corresponded to PWS or was non-significantly different in some variables that were considered here as potential determinants of PWS. Namely, mechanical efficiency, fat and carbohydrate oxidation and stability parameters met the criteria, and it is believed that participants chose their PWS based on these factors. A strength of the present study was its sample size (N = 34) as compared to other similar studies that examined 11 (Jordan et al., 2007), 12 (Willis et al., 2005), 14 (Godsiff et al., 2018), or 23 participants (Fernández Menéndez et al., 2019). In addition, the simultaneous examination of metabolic, perceptual, spatiotemporal and stability parameters at an absolute speed range around the individual PWS is also innovative as it brings a new perspective to studying the relationship between studied variables and speed. To our knowledge, no previous studies have done that, and the closest studies have examined different speeds

presented as a percentage of PWS which makes it difficult to relate to actual magnitude of change in speed when examining relationships with intensity. Moreover, this also makes it harder to recommend a walking exercise on treadmill. Findings allow us to predict for a sedentary young adult an average of 110 kcal spent in a 30-min walk at PWS. The best fit equations (Table 4) could be used to predict a further rise of 11 kcal for a 30-min walk at PWS+0.5 km·h^{-1} or an additional 43 kcal at PWS+1.5 km·h^{-1}. The proportion of this energy expended coming from fat oxidation can also be predicted using the equations.

One weakness of the present study is that walking was performed on a treadmill rather than overground, and in a controlled laboratory environment. While most studies have used a treadmill to acquire larger number of gait cycles or due to other limitations (e.g., equipment), walking on a treadmill seems to be more stable and less variable (Dingwell et al., 2001). However, both treadmill and overground locomotion do not seem to present mechanical differences (van Ingen Schenau, 1980). Walking in a natural outdoor environment rather than a controlled laboratory one also confers additional psychological benefits that were not accounted for in the present study (Focht, 2009). Indeed, in an outdoor environment and as compared to a laboratory environment, preferred walking speed is expected to be higher and ratings of perceived exertion lower (Focht, 2009). Another weakness of the current study relates to the duration of speed trials that only allow prediction of short-term acute responses to walking. Even though a steady state was reached at each trial (given the rather low-intensity nature of all trials), potential changes in various responses with time are to be investigated in future studies. Furthermore, similar future study designs should include maximal exercise testing to account for maximal aerobic capacity and anaerobic thresholds, which could provide a better understanding of the relative effort and intensity reached at PWS, compared to lower and higher speeds. This would help to obtain insights into whether walking at PWS occurs within general physical activity intensity recommendations. Indeed, de Moura et al. (2011) have verified that most normal weight individuals adopt a PWS that falls into the "moderate" intensity classification and was judged adequate to elicit health benefits according to ACSM recommendations.

In sum, the present findings support benefits of walking at the preferred walking speed for sedentary young adults, as opposed to walking at a slower or faster prescribed intensity. The choice of the preferred walking speed seems to be determined by factors being optimized at that intensity and related to mechanical efficiency, stability of gait and fuel oxidation. Therefore, walking at one's preferred intensity could be an effective strategy for young sedentary adults to become active. Given the increased fat oxidation at the preferred intensity, walking at PWS could also serve as a safe exercise for weight management or disease prevention. Future studies could focus on examining responses to preferred walking for different portions of healthy and clinical populations. Finally, it is recommended that physical activity guidelines focus less on prescribing intensities and more on encouraging self-determined active behaviors and intensities, not just for their long-term benefits on adherence and overall health, but also for their potential acute benefits even when performed in short bouts.

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 humans were approved by the Qatar University's Institutional Review Board (IRB), Qatar University, Doha, Qatar. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

LM: Conceptualization, Data curation, Formal Analysis, Methodology, Supervision, Writing–original draft, Writing–review and editing. RI: Formal Analysis, Methodology, Writing–review and editing. ML: Writing–review and editing. GJ: Conceptualization, Writing–review and editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The publication of this article was funded by the Qatar National Library.

Acknowledgments

The authors would like to acknowledge the efforts of Abderrahman Ouattas, Wajiha Ghazi, Rania Bouhesine, Mohamed Riahi and Mohamed Elhams in data collection.

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.

Publisher's note

References

Abernethy, B., Hanna, A., and Plooy, A. (2002). The attentional demands of preferred and non-preferred gait patterns. *Gait Posture* 15 (3), 256–265. doi:10.1016/s0966-6362(01)00195-3

Achten, J., and Jeukendrup, A. E. (2004). Optimizing fat oxidation through exercise and diet. *Nutrition* 20 (7-8), 716–727. doi:10.1016/j.nut.2004.04.005

Alexander, R. M. (2002). Energetics and optimization of human walking and running: the 2000 Raymond Pearl memorial lecture. *Am. J. Hum. Biol.* 14 (5), 641–648. doi:10.1002/ajhb.10067

Beauchet, O., Allali, G., Annweiler, C., Bridenbaugh, S., Assal, F., Kressig, R. W., et al. (2009). Gait variability among healthy adults: low and high stride-to-stride variability are both a reflection of gait stability. *Gerontology* 55 (6), 702–706. doi:10.1159/000235905

Bohannon, R. W., and Andrews, A. W. (2011). Normal walking speed: a descriptive meta-analysis. *Physiotherapy* 97 (3), 182–189. doi:10.1016/j.physio.2010.12.004

Bohannon, R. W., and Wang, Y. C. (2019). Four-meter gait speed: normative values and reliability determined for adults participating in the NIH toolbox study. *Archives Phys. Med. Rehabilitation* 100 (3), 509–513. doi:10.1016/j.apmr.2018.06.031

Borg, G. A. V. (1973). Perceived exertion: a note on "history" and methods. *Med. Sci. Sports* 5, 90–93. doi:10.1249/00005768-197300520-00017

Brouwer, E. (1957). On simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat oxidized in metabolism of men and animals, from gaseous exchange (Oxygen intake and carbonic acid output) and urine-N. *Acta Physiologica Pharmacol. Neerl.* 6, 795–802.

Brown, D. (2015). *Tracker (version 4.91)*. software. Open Source Physics.

Carnethon, M., Whitsel, L. P., Franklin, B. A., Kris-Etherton, P., Milani, R., Pratt, C. A., et al. (2009). Worksite wellness programs for cardiovascular disease prevention: a policy statement from the American Heart Association. *Circulation.* 120 (17), 1725–1741.

Chung, M. J., and Wang, M. J. J. (2010). The change of gait parameters during walking at different percentage of preferred walking speed for healthy adults aged 20–60 years. *Gait Posture* 31 (1), 131–135. doi:10.1016/j.gaitpost.2009.09.013

Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., et al. (2003). International physical activity questionnaire: 12-country reliability and validity. *Med. Sci. Sports Exerc.* 35 (8), 1381–1395. doi:10.1249/01.MSS.0000078924.61453.FB

Dal, U., Erdogan, T., Resitoglu, B., and Beydagi, H. (2010). Determination of preferred walking speed on treadmill may lead to high oxygen cost on treadmill walking. *Gait Posture* 31, 366–369. doi:10.1016/j.gaitpost.2010.01.006

de Moura, B. P., Marins, J. C. B., and Amorim, P. R. S. (2011). Self selected walking speed in overweight adults: is this intensity enough to promote health benefits? *Apunts. Med. l'Esport* 46 (169), 11–15. doi:10.1016/j.apunts.2010.10.004

Diedrich, F. J., and Warren, W. H., Jr (1995). Why change gaits? Dynamics of the walk-run transition. *J. Exp. Psychol. Hum. Percept. Perform.* 21 (1), 183–202. doi:10.1037//0096-1523.21.1.183

Dingwell, J. B., Cusumano, J. P., Cavanagh, P. R., and Sternad, D. (2001). Local dynamic stability versus kinematic variability of continuous overground and treadmill walking. *J. Biomechanical Eng.* 123 (1), 27–32. doi:10.1115/1.1336798

di Prampero, P. E. (1986). The energy cost of human locomotion on land and in water. *Int. J. Sports Med.* 7, 55–72. doi:10.1055/s-2008-1025736

Eaton, S. B., and Eaton, S. B., III (2003). An evolutionary perspective on human physical activity: implications for health. *Comp. Biochem. Physiology Part A Mol. Integr. Physiology* 136 (1), 153–159. doi:10.1016/s1095-6433(03)00208-3

Ekkekakis, P., Backhouse, S. H., Gray, C., and Lind, E. (2008). Walking is popular among adults but is it pleasant? A framework for clarifying the link between walking and affect as illustrated in two studies. *Psychol. Sport Exerc.* 9 (3), 246–264. doi:10.1016/j.psychsport.2007.04.004

Ekkekakis, P., Parfitt, G., and Petruzzello, S. J. (2011). The pleasure and displeasure people feel when they exercise at different intensities: decennial update and progress towards a tripartite rationale for exercise intensity prescription. *Sports Med.* 41 (8), 641–671. doi:10.2165/11590680-000000000-00000

Fernández Menéndez, A., Saubade, M., Hans, D., Millet, G. P., and Malatesta, D. (2019). The determinants of the preferred walking speed in individuals with obesity. *Obes. facts* 12 (5), 543–553. doi:10.1159/000501968

Fischer, G., de Queiroz, F. B., Berton, D. C., Schons, P., Oliveira, H. B., Coertjens, M., et al. (2021). Factors influencing self-selected walking speed in fibrotic interstitial lung disease. *Sci. Rep.* 11 (1), 12459. doi:10.1038/s41598-021-91734-x

Focht, B. C. (2009). Brief walks in outdoor and laboratory environments: effects on affective responses, enjoyment, and intentions to walk for exercise. *Res. Q. Exerc. Sport* 80, 611–620. doi:10.1080/02701367.2009.10599600

Garby, L., and Astrup, A. (1987). The relationship between the respiratory quotient and the energy equivalent of oxygen during simultaneous glucose and lipid oxidation and lipogenesis. *Acta Physiol. Scand.* 129 (3), 443–444. doi:10.1111/j.1365-201x.1987.tb10613.x

Godsiff, D. T., Coe, S., Elsworth-Edelsten, C., Collett, J., Howells, K., Morris, M., et al. (2018). Exploring the metabolic and perceptual correlates of self-selected walking speed under constrained and un-constrained conditions. *J. Sports Sci. Med.* 17 (1), 1–6.

Haight, D. J., Lerner, Z. F., Board, W. J., and Browning, R. C. (2014). A comparison of slow, uphill and fast, level walking on lower extremity biomechanics and tibiofemoral joint loading in obese and nonobese adults. *J. Orthop. Res.* 32 (2), 324–330. doi:10.1002/jor.22497

Hamacher, D., Singh, N. B., Van Dieën, J. H., Heller, M. O., and Taylor, W. R. (2011). Kinematic measures for assessing gait stability in elderly individuals: a systematic review. *Journal of The Royal Society Interface* 8 (65), 1682–1698.

Hansen, C., Einarson, E., Thomson, A., Whiteley, R., and Witvrouw, E. (2017). Hamstring and calf muscle activation as a function of bodyweight support during treadmill running in ACL reconstructed athletes. *Gait Posture* 58, 154–158. doi:10.1016/j.gaitpost.2017.07.120

Holt, K. G., Hamill, J., and Andres, R. O. (1990). The force-driven harmonic oscillator as a model for human locomotion. *Hum. Mov. Sci.* 9 (1), 55–68. doi:10.1016/0167-9457(90)90035-c

Holt, K. G., Hamill, J., and Andres, R. O. (1991). Predicting the minimal energy costs of human walking. *Med. Sci. Sports Exerc.* 23 (4), 491–498. doi:10.1249/00005768-199104000-00016

Huynh, H., and Feldt, L. S. (1970). Conditions under which mean square ratios in repeated measurements designs have exact F-distributions. *J. Am. Stat. Assoc.* 65, 1582–1589. doi:10.1080/01621459.1970.10481187

Jeng, S. F., Liao, H. F., Lai, J. S., and Hou, J. W. (1997). Optimization of walking in children. *Med. Sci. Sports Exerc.* 29 (3), 370–376. doi:10.1097/00005768-199703000-00012

Jordan, K., Challis, J. H., and Newell, K. M. (2007). Walking speed influences on gait cycle variability. *Gait Posture* 26 (1), 128–134. doi:10.1016/j.gaitpost.2006.08.010

Klein, S., Coyle, E. F., and Wolfe, R. R. (1994). Fat metabolism during low-intensity exercise in endurance-trained and untrained men. *Am. J. Physiol. Endocrinol.* 267 (6), 934–940.

Lafortuna, C. L., Proietti, M., Agosti, F., and Sartorio, A. (2006). The energy cost of cycling in young obese women. *Eur. J. Appl. Physiology* 97, 16–25. doi:10.1007/s00421-006-0137-5

Majed, L., Hansen, C., and Girard, O. (2020). Preferred gait characteristics in young adults in Qatar: physiological, perceptual, and spatiotemporal analysis. *Sage Open* 10 (3), 215824402094572. doi:10.1177/2158244020945721

Majed, L., Sayegh, S., and Chrismas, B. C. (2022). Reference walking speeds for healthy young adults in Qatar: moderating effect of obesity and physical activity. *Sage Open* 12 (1), 215824402210799. doi:10.1177/21582440221079919

Margaria, R. (1976). *Biomechanics and energetics of muscular exercise*. Oxford, UK: Oxford University Press.

McGilvery, R. W., and Goldstein, G. W. (1983). *Biochemistry: a functional approach*. WB Saunders.

Medbo, J. I., Mamen, A., Welde, B., Von Heimburg, E., and Stokke, R. (2002). Examination of the Metamax I and II oxygen analysers during exercise studies in the laboratory. *Scand. J. Clin. Laboratory Investigation* 62, 585–598. doi:10.1080/00365102764654321

Medbo, J. I., and Tabata, I. (1989). Relative importance of aerobic and anaerobic energy release during short-lasting exhausting bicycle exercise. *J. Appl. Physiology* 67, 1881–1886. doi:10.1152/jappl.1989.67.5.1881

Meyer, C., Killeen, T., Easthope, C. S., Curt, A., Bolliger, M., Linnebank, M., et al. (2019). Familiarization with treadmill walking: how much is enough? *Sci. Rep.* 9 (1), 5232. doi:10.1038/s41598-019-41721-0

Özdemir, Ç., Özgünen, K., Günaştı, Ö., Eryılmaz, S. K., Kılcı, A., and Kurdak, S. S. (2019). Changes in substrate utilization rates during 40 min of walking within the Fatmax range. *Physiol. Int.* 106 (3), 294–304. doi:10.1556/2060.106.2019.28

Parfitt, G., and Hughes, S. (2009). The exercise intensity–affect relationship: evidence and implications for exercise behavior. *J. Exerc. Sci. Fit.* 7 (2), 34–41. doi:10.1016/s1728-869x(09)60021-6

Peronnet, F., and Massicotte, D. (1991). Table of nonprotein respiratory quotient: an update. *Can. J. Sport Sci.* 16 (1), 23–29.

Perri, M. G., Anton, S. D., Durning, P. E., Ketterson, T. U., Sydeman, S. J., Berlant, N. E., et al. (2002). Adherence to exercise prescriptions: effects of prescribing moderate versus higher levels of intensity and frequency. *Health Psychol.* 21 (5), 452–458. doi:10.1037//0278-6133.21.5.452

Peters, J. C., Wyatt, H. R., Donahoo, W. T., and Hill, J. O. (2002). From instinct to intellect: the challenge of maintaining healthy weight in the modern world. *Obes. Rev.* 3 (2), 69–74. doi:10.1046/j.1467-789x.2002.00059.x

Ralston, H. J. (1958). Energy-speed relation and optimal speed during level walking. *Int. Z. fur Angew. Physiol.* 17, 277–283. doi:10.1007/BF00698754

Ravussin, E., Bogardus, C., Scheidegger, K., LaGrange, B., Horton, E. D., Horton, E. S., et al. (1986). Effect of elevated FFA on carbohydrate and lipid oxidation during prolonged exercise in humans. *J. Appl. Physiol.* 60 (3), 893–900.

Saibene, F., and Minetti, A. E. (2003). Biomechanical and physiological aspects of legged locomotion in humans. *Eur. J. Appl. Physiology* 88, 297–316. doi:10.1007/s00421-002-0654-9

Sparrow, W. A., and Newell, K. M. (1998). Metabolic energy expenditure and the regulation of movement economy. *Psychonomic Bull. Rev.* 5, 173–196. doi:10.3758/bf03212943

van Ingen Schenau, G. (1980). Some fundamental aspects of the biomechanics of overground versus treadmill locomotion. *Medicine and Science in Sports and Exercise* 12 (4), 257–261.

Williams, D. M. (2008). Exercise, affect, and adherence: an integrated model and a case for self-paced exercise. *J. Sport and Exerc. Psychol.* 30 (5), 471–496. doi:10.1123/jsep.30.5.471

Williams, D. M., Dunsiger, S., Miranda, R., Jr, Gwaltney, C. J., Emerson, J. A., Monti, P. M., et al. (2015). Recommending self-paced exercise among overweight and obese adults: a randomized pilot study. *Ann. Behav. Med.* 49 (2), 280–285. doi:10.1007/s12160-014-9642-7

Willis, W. T., Ganley, K. J., and Herman, R. M. (2005). Fuel oxidation during human walking. *Metabolism* 54, 793–799. doi:10.1016/j.metabol.2005.01.024

Zarrugh, M. Y., Todd, F. N., and Ralston, H. J. (1974). Optimization of energy expenditure during level walking. *Eur. J. Appl. Physiology Occup. Physiology* 33, 293–306. doi:10.1007/BF00430237

frontiers | Frontiers in Physiology

TYPE Original Research
PUBLISHED 12 March 2024
DOI 10.3389/fphys.2024.1365357

OPEN ACCESS

EDITED BY
Cristina Vassalle,
Gabriele Monasterio Tuscany Foundation (CNR), Italy

REVIEWED BY
Annamaria Mancini,
University of Naples Parthenope, Italy
Pasqualina Buono,
Università degli Studi di Napoli Parthenope, Italy

*CORRESPONDENCE
Lise Bjørkhaug,
lise.bjorkhaug.gundersen@hvl.no

†These authors have contributed equally to this work and share first authorship

RECEIVED 04 January 2024
ACCEPTED 26 February 2024
PUBLISHED 12 March 2024

CITATION
Ryningen A, Rostad K, Ersvær E, Sjøholt G, Paulsen G, Gundersen H, Kristoffersen M and Bjørkhaug L (2024), Acute response in circulating microRNAs following a single bout of short-sprint and heavy strength training in well-trained cyclists.
Front. Physiol. 15:1365357.
doi: 10.3389/fphys.2024.1365357

Acute response in circulating microRNAs following a single bout of short-sprint and heavy strength training in well-trained cyclists

Anita Ryningen[1†], Kari Rostad[1†], Elisabeth Ersvær[1,2], Gry Sjøholt[1], Gøran Paulsen[3,4], Hilde Gundersen[5], Morten Kristoffersen[5] and Lise Bjørkhaug[1*]

[1]Department of Safety, Chemistry and Biomedical Laboratory Sciences, Western Norway University of Applied Sciences, Bergen, Norway, [2]Department of Biotechnology, Inland Norway University of Applied Sciences, Lillehammer, Norway, [3]Department of Sport, Food and Natural Sciences, Western Norway University of Applied Sciences, Sogndal, Norway, [4]Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, Norway, [5]Department of Sport and Physical Activity, Western Norway University of Applied Sciences, Bergen, Norway

Background: Heavy strength (HS) and short-sprint (SS) are commonly used training methods for competitive road cyclists, with the aim to improve the anaerobic power and short time cycling performance. Knowledge of how such training methods affects biochemical as well as molecular factors, are particularly important for determining individual recovery and long-term adaptations. The primary aim of the current study was to investigate the expression levels of small non-coding RNAs in response to HS and SS training in elite cyclists as potential biomarkers for individual optimal restitution time.

Methods: Eleven well trained cyclists performed one session of HS training and one session of SS training on separate days. Blood samples were taken at baseline and 5 min, 1 h and 21 h post training. Along with physiological measurements and biochemical factors (serum creatine kinase, myoglobin, human growth hormone and plasma lactate), real-time quantitative PCR was used to explore whether HS and/or SS training influenced the abundance of 24 circulating miRNAs, in serum, associated with muscle development, angiogenesis, and/or inflammation.

Results: Based on complete miRNA profiles from nine cyclists, the miRNAs showing most altered expression after both training sessions included the three striated muscle-specific miRNAs (myomiRs) miR-1-3p, 133a-3p and 133b-3p. While all three miRNAs showed significantly highest expression at 1 h post HS session, the acute effect of the SS session included a significantly higher level of miR-1-3p alone, at 5 min (highest), as well as at 1 h and 21 h post session. Correlation (negative) with biochemical markers was only shown for miR-133a-3p and CK ($r = -0.786$, $p = 0.041$) and between miR-133b-3p and [La^-] ($r = -0.711$, $p = .032$), at 21 h post SS session.

Conclusion: Our findings support that unique myomiRs are regulated by HS and SS training. Such knowledge may be important for individually adjusted restitution times.

KEYWORDS

cycling, heavy strength training, short-sprint training, MicroRNAs, recovery

Introduction

Competitive road cycling is an endurance sport which demands sustained aerobic power outputs (Jeukendrup et al., 2000). However, high-intensity efforts and sprinting abilities are important to manage sudden breakaways, to close gaps to riders ahead, and in a fast finishing sprint (Menaspa et al., 2015). Thus, many cyclists also include heavy strength (HS) training and specific short sprint (SS) training, with the aim of improving sprinting power.

Both HS and SS training challenges the neuromuscular system to generate maximal power, by different combinations of load and velocity. While HS is performed with high loads and slow concentric and eccentric muscle actions, cycling-specific SS training applies lower load and higher velocity muscle contractions (Kraemer et al., 2004; Martin, 2007). Although the effects of HS training on cycling performance have long been debated, an increase in maximal force (Bertucci et al., 2005; Skovereng et al., 2018; Del Vecchio et al., 2019) and muscle hypertrophy (Ronnestad et al., 2010; Vikmoen et al., 2016) has been shown in trained cyclists, after adding HS training to their usual endurance training, with positive effects on anaerobic power and short time cycling performance. Unlike HS training, the effect of SS training on cycling performance has been less studied, possibly due to the lack of well-established training methods and debate on how to measure sprint performance. Knowledge regarding the acute effect of systematic HS and SS training on sprint performance, aerobic capacity and recovery time, is undoubtedly valuable for both athletes, coaches and researchers. It is also important to gain knowledge about physiological, biochemical and metabolic factors involved in individual recovery and long-term adaptions after such training.

Exercise *per se* induce changes in gene expression, which affect skeletal and myocardial metabolism and regeneration, as well as mitochondrial synthesis, angiogenesis and inflammation (Gundersen, 2011; Egan and Zierath, 2013; Camera et al., 2016; Hughes et al., 2018). One of the molecular co-players in gene expression and modulation of signal propagation are the microRNAs (miRNA). MiRNAs are small (19–22 nucleotides), endogenous, non-coding RNA molecules that regulate gene expression by repressing specific target genes either by translational inhibition or mRNA degradation (Krol et al., 2010). The potential role of miRNAs in communication between cells and tissues is strongly supported by the fact that miRNAs can be exported and imported by cells through mechanisms involving vesicle trafficking and protein carriers. Circulating blood plasma miRNAs (c-miRNA) are associated with microparticles (Rayner and Hennessy, 2013), exosomes (Valadi et al., 2007), apoptotic bodies (Zernecke et al., 2009), proteins (e.g., Argonaute) (Li et al., 2012), or high-density lipoproteins (Vickers et al., 2011). Further, miRNAs are actively or passively secreted into human biofluids where they can have systemic effects (Kosaka et al., 2013; Shah and Calin, 2013). As miRNAs are quite stable molecules (Tsui et al., 2002; Mitchell et al., 2008) and easily accessible via blood samples, c-miRNAs represent interesting biological markers associated with the physiological responses to exercise and adaption (Horak et al., 2018; Sapp et al., 2019). Circulating miRNA are found to be regulating proliferation, differentiation, hypertrophy, and nutrient metabolism in skeletal muscle functions (Domanska-Senderowska et al., 2019), in angiogenesis, inflammation (Li et al., 2018), and in metabolic change (Massart et al., 2021). Muscle induced c-miRNAs and c-miRNA biomarkers for endothelial damage have been shown altered in response to acute aerobic exercise (Nielsen et al., 2014; Uhlemann et al., 2014). Also, c-miRNAs playing a role in strength training-induced muscle adaptation and as biomarkers for muscle damage have been shown altered in response to resistance exercise session (Sawada et al., 2013; Uhlemann et al., 2014).

In a recent study, we found that the acute responses following HS and SS training sessions in trained cyclists differ, whereby such training sessions differentially affect physiological and biochemical markers of metabolic stress and muscle damage, in serum, relevant for individual recovery and long-term adaptations (Kristoffersen et al., 2019). In this study, we thus followed up by investigating miRNAs associated with metabolic homeostasis. More specifically, 24 c-miRNAs with expected functional regulation in skeletal and heart muscle, hypoxia and angiogenesis, and in metabolism and inflammation, listed in Supplementary Table S1, were profiled from the same homogeneous group of young male elite cyclists (Kristoffersen et al., 2018), acutely after performing a SS and an HS training session. We aimed to explore (1) differences in c-miRNA profile responses to SS and HS training sessions, and (2) correlation between unique c-miRNAs and biochemical markers of muscle metabolism and damage (lactate, human growth hormone, creatine kinase and myoglobin). Our major/overall goal was to evaluate specific c-miRNAs as potential markers of recovery for long-term adaptions to training in well-trained athletes, based on their acute response post HS and SS training.

Materials and methods

Participants

Eleven well-trained cyclists (males), adept with HS and SS training from daily training routines, gave their written informed consent to participate in the study. Criteria fulfilled for participation included: i) doing competitive cycling at national or international level, ii) having maximal oxygen uptake (VO_{2max}) of ≥60 mL · kg^{-1} · min^{-1}, iii) having implemented strength training, including squat, hip flexion and leg press, twice a week for a minimum of 4 weeks before testing, and iv) being currently healthy and free from injury. Baseline physiological characteristics of nine of the eleven study

TABLE 1 Physiological characteristics of the male cyclists included in the study (n = 9).

Age (years)	17.8 ± 1.7
Body height (cm)	182.2 ± 6.6
Body mass (kg)	72.0 ± 4.7
Body fat (%)	11.4 ± 3.2
VO_2max (ml · kg^{-1} min^{-1})	66.4 ± 2.5
Power output at [La−] of 4 mmol·L^{-1} (W)	285 ± 25

participants where reliable and shown in Table 1, and complete miRNA profiles were provided. The study was evaluated by The Regional Committee for Medical and Health Research Ethics in West Norway to not include any medical or health related ethical concerns. The study was then approved by The Norwegian Data Protection Authority (45048).

Study design

The study was performed with a crossover design. Participants were randomly divided into two groups, and the HS and SS training sessions were conducted in a randomized order. 48 h passed between the two sessions for both groups. Blood samples were collected before breakfast (baseline/0 h) and at 5 min, 1 h and 21 h post training sessions (Figure 1).

Preliminary testing and familiarization session

Approximately 14 days before the first experimental training session, a submaximal and maximal test on a cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) was completed by the participants, as described by Kristoffersen et al. (2018). Familiarization to the specific sessions used in the present study was performed 1 week before the first experimental session. The 6RM load for each exercise in the HS session was defined during the familiarization session for each participant. During the cycling familiarization session, the pedaling resistance applied for the sprints was individually adjusted using an air braked bicycle ergometer (WattBike, WattBike Ltd, Nottingham, UK). This bike was used for both familiarization and experimental trials. To ensure that the participant achieved the highest possible power output during the 8-s sprints at a cadence of 130–140 revolutions per min (RPM) (Hopker et al., 2010), each participant performed at least three sprint at different resistance level with 2 min recovery in between.

Experimental procedures

In order to perform the same volume of low-intensity training during the data collection period, all participants were instructed to abstain from strenuous exercise. They were also instructed to have similar diet 48 h prior to both experimental training sessions. All participants registered daily meals, physical activity and sleep, during the data collection period. Prior to the HS and SS training sessions, individuals arrived to the laboratory for the baseline blood sample at the same daily time after an overnight fast of at least 8 h. Participants were served a standardized breakfast 1.5 h prior to each training session.

HS and SS training sessions

The HS and SS sessions were carefully designed to mirror typical training sessions used by Norwegian world-class cyclists. The total duration of the SS and HS session were approximately 45 min including warm-up.

The SS session consisted of three sets of four 8 s intervals with maximal effort, from a standstill start in a seated position (individually chosen preferred leg). Each interval repetition was spaced by a 2 min active recovery, and each sprint set by a 5 min active recovery. Active recovery included cycling at 70% of HR_{max}. A cycle ergometer (WattBike, WattBike Ltd, Nottingham, UK) was used for all SS sessions, and allowed measurement of power output (Hopker et al., 2010).

The HS sessions included 3 sets of 6 repetition maximum (RM) per exercise. Sessions consisted of two-legged squats in a smith machine (TKO, Houston, USA), unilateral leg-press (Mobility, Norway), and unilateral hip flexor exercises in a cable cross apparatus (Gym 80, Gleskirchen, Deutschland). Three min recovery was given between sets and 5 min between each

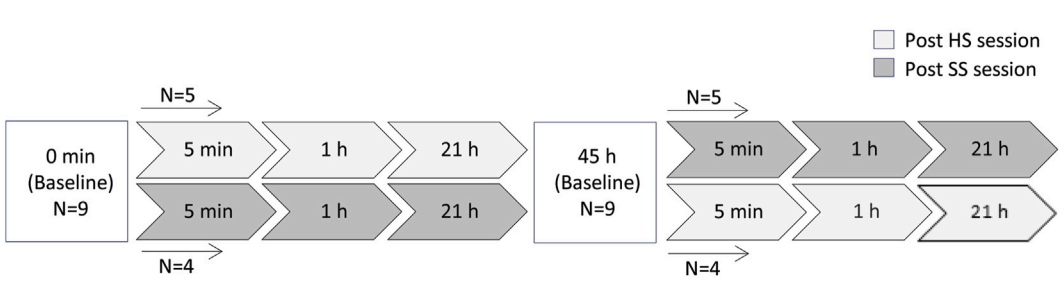


FIGURE 1
An overview of the study design/experimental setup and timepoints pre/post HS and SS training sessions. N indicates the number of individuals performing in each training session. HS and SS indicate heavy strength training and specific short sprint training, respectively.

exercise. Participants were instructed to carry out the concentric phase with maximal effort. The eccentric phase was instructed completed as a controlled movement lasting for 2 s.

Blood sampling and serum analyses

Blood samples were collected pre-exercise (baseline, in fasted state) and at 5 min, 1 h, and 21 h post the HS and SS training session. Blood was sampled from an antecubital vein into vacutainers containing clot activator for serum separation (BD Vacutainer SST II Advance Plus Blood Collection Tubes) and were kept at room temperature for 30 min prior to centrifugation (1,300 x g for 10 min). Serum for miRNA analyses was aliquoted to Nunc cryotubes and subsequently stored at −80°C until analyzed.

Biochemical marker levels

Serum creatine kinase (CK), myoglobin (Mb), human growth hormone (hGH) and plasma lactate ([La]) was analyzed as previously described (Kristoffersen et al., 2018). Biochemical marker levels at specific time points post training session are presented in Supplementary Figure S2.

TaqMan low density array (TLDA) assay

For the TLDA assay, total RNA was extracted from 100 µL serum samples using the MagMAX *mir*VANA Total RNA Isolation Kit (Applied Biosystems, Foster City, CA) and according to the manufacturers' instructions. Eluted RNA (50 µL) was stored at −20°C until further analysis. For oligo RNA spike-in controls (oligo-cel-miR-54-3p, -238-3p and -39-3p), 5 µL 0.2 nM spike-in RNA oligos was added to 100 µL serum samples to a final concentration of 10 pM prior to RNA extraction. cDNA synthesis was performed using the TaqMan Advanced miR cDNA synthesis kit (Applied Biosystems, Foster City, CA) and according to the manufacturers' instructions. Mature miRNAs were firstly modified in a poly(A) tailing reaction using 2 µL total RNA in the reaction mix (0.5 µL 10X Poly(A) buffer, 0.5 µL ATP, 0.3 µL Poly(A) enzyme, and 1.7 µL nuclease free water), followed by adding 5 µL into the subsequent adaptor ligation reaction mix (3 µL 5X DNA Ligase buffer, 4.5 µL 50% PEG 8000, 0.6 µL 25X Ligation Adaptor, 1.5 µL RNA Ligase, and 0.4 µL nuclease free water). For the reverse transcription reaction, equal volumes (15 µL) of reaction mix (6 µL 5X RT Buffer, 1.2 µL 25 mM dNTPs, 1.5 µL 20X Universal RT Primer, 3.0 µL 10XRT Enzyme Mix, and 3.3 µL nuclease free water) and adapter ligation reaction product was used.

miRNA profiling was performed using the TLDA custom-configured TaqMan Array Cards (Applied Biosystems, Waltham, USA). In order to increase the sensitivity of the TLDA, a preamplification was performed after the reverse transcription using a miR-Amp reaction mix (25 µL 2X miR-Amp Master Mix, 2.5 µL 20X miR-Amp Primer Mix, and 17.5 µL nuclease free water) with 5 µL modified mature miRNAs. For the real-time PCR reactions, 25 µL of a 1:10 dilution of the cDNA template (miR-Amp reaction product) was used in the PCR reaction mix also including 50 µL 2X TaqMan Fast Advanced Mastermix and 25 µL RNase free water. 100 µL PCR reaction mix was applied to each port of the TLDA-card resulting in a 1 µL volume in each well.

The 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) was used for the real-time PCR run and all reactions were performed as specified in the protocol of the manufacturer (TaqMan Advance miRNA assays User Guide. TaqMan Array Cards (MAN0016122)). Properties of the run was Comparative Ct (ΔΔCt) and fast cycling mode. miRNA concentrations were analyzed using the ExpressionSuite software (Applied Biosystems, Foster City, CA) and for the normalization of miRNA expression levels, the average C_t values of all miRNAs (global normalization) was used. The fold changes of miRNA expression were calculated using the eq. 2-ΔΔCq.

Statistical analyses and software

To obtain comprehensive miRNA expression profiles at various time points following the HS and SS training sessions, we examined the expression profiles of 24 miRNAs in blood serum from the nine elite cyclists. To identify differences in the expressions of miRNAs in the training session groups, compared to baseline expression, we performed a volcano plot filtering against these miRNA expression profiles at specific post session time points. Clustering analysis of the expression patterns of microRNAs was performed using ExpressionSuite software.

One-way ANOVA analyses were performed on the LOG (2^-(ΔΔ-Ct)) values to evaluate differences between baseline and 5 min, 1 h and 21 h following both the HS and the SS training session for each of the three miRNAs miR-1-3p, miR-133a-3p and miR-133b-3p. (A Shapiro-Wilk analysis was firstly performed and confirmed that the data were normally distributed). Least Significant Difference (LSD) *post hoc* tests used for further comparisons.

Relationships between levels of miRNAs and biochemical markers ([La], CK, Mg, hGH) after HS and SS training sessions were assessed using Pearson's correlation coefficient analysis (Batterham and Hopkins, 2006). An r-value between .01 and .29 was defined as a small correlation, between 0.30 and 0.49 as a medium correlation, and from 0.5 to 1.0 as a large correlation (Cohen, 1988). The Statistical Products of Service Solution package (SPSS Statistics, version 29) was used for all statistical analyses. For all analyses, statistical significance was determined as <0.05. For the correlation analyses, confidence intervals (CI) are presented.

Results

Differentially expressed miRNAs after HS and SS training sessions

The overall expression profiles of the 24 serum miRNAs in the nine elite cyclists was firstly performed by unsupervised hierarchical clustering of all miRNAs, for all timepoints after the SS and HS training sessions (Supplementary Figure S1). We followed up by investigating levels of three unique miRNAs expressions at individual time points after HS and SS training sessions (Figure 2).

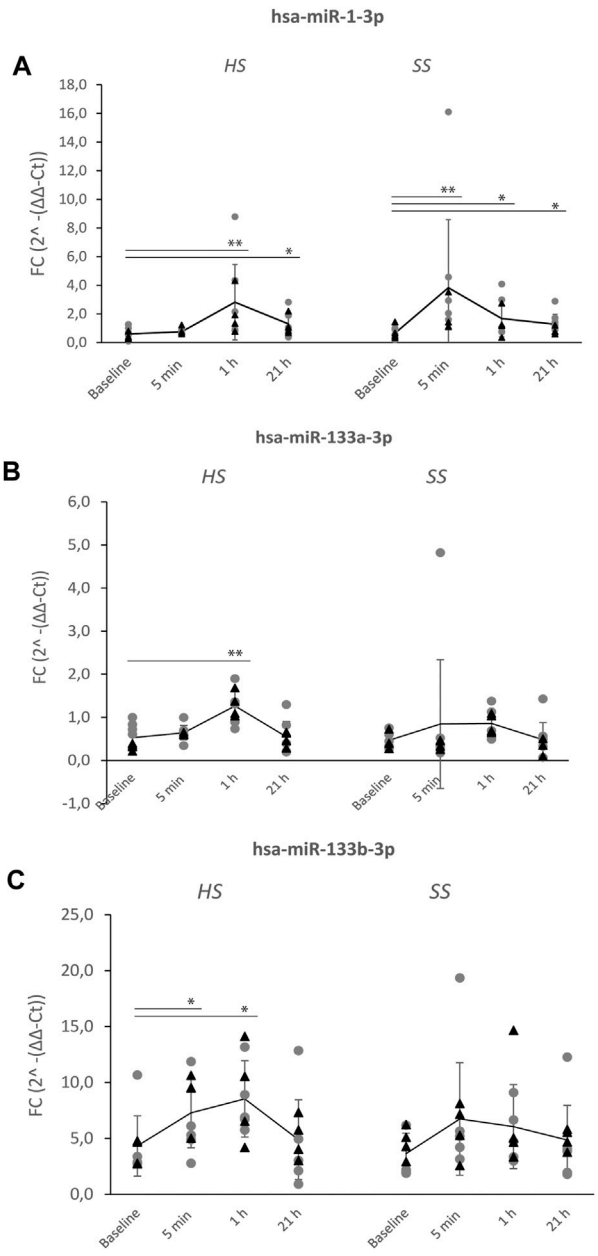


FIGURE 2
Levels of upregulated hsa-miR-1-3p, hsa-miR-133a-3p and hsa-miR-133b-3p after the HS and SS training sessions. Serum concentrations of three miRNAs were measured following a heavy strength training session (HS) and a short-sprint training session (SS) pre-(baseline) and post-exercise (5 min, 1 h and 21 h) in nine well-trained cyclists (Figure 1). Significant differences after HS and SS sessions of **(A)** hsa-miR-1-3p, **(B)** hsa-miR-133a-3p, **(C)** hsa-miR-133b-3p, compared to baseline levels, are indicated as $*p < 0.05$, $**p < 0.01$. Mean levels (line-graph) and standard deviations (vertical lines) are shown for each time point. Gray dots (●) shows miRNA levels of individuals starting with HS > SS session, while black triangles (▲) shows miRNA levels of individuals starting with SS > HS session.

One-way ANOVA analyses showed significant main effects in Fold Change $2^{-}(\Delta\Delta\text{-}Ct)$ values for the three miRNAs comparing before and after the HS training session (has-miR-1-3p: $F(3,32) = 7.469$, $p < 0.01$, has-miR-133a-3p: $F(3,32) = 8.828$, $p < 0.01$ and has-miR-133b-3p: $F(3,31) = 4.068$, $p < 0.015$). A *post hoc* (LSD) test showed significantly higher levels of all three miRNAs at 1 h post HS session (has-miR-1-3p and hsa-miR-133a-3p by $p < 0.01$ and has-miR-133b-3p by $p = 0.011$). Has-miR-1-3p levels alone was also significantly increased at 21 h post HS session ($p = 0.013$) (Figures 2A–C).

A significant main effect before and after the SS training session was only found for has-miR-1-3p ($F(3,32) = 7.115$, $p < 0.001$). The *post hoc* test showed significantly higher levels of this miRNA already at 5 min ($p < 0.001$), at 1 h ($p < 0.015$) and at 21 h ($p = 0.035$).

Analysis of altered expression patterns of the 24 miRNAs (including the 4 control miRNAs and 3 spike-in miRNAs) at various time points post HS and SS training session is also presented by Volcano plots (Figure 3), by their $\log_2$(Fold Change), compared (normalized) to session specific baseline miRNA levels. A change in miRNA expression was considered statistically significant if the fold change (FC) was >2.0 and the *p*-value was <0.05.

By this, we found that miR-1-3p demonstrated the largest fold change (FC of 4.3; $p = 0.01$) at 5 min post the SS training session, based on miR-1-3p $\log_2$(Fold Change) values of 2,12 (Figure 3). Upregulated miR-1-3p levels were then reduced to FC = 2.2 ($p = 0.042$) at 1 h and FC = 1.8 ($p = 0.042$) at 21 h post the SS training session (Figure 3). Following the HS training session, miR-1-3p demonstrated a FC of 4.02 ($p = 0.008$) at 1 h and non-significant FC of 2.3 at 21 h, compared to baseline (Figure 3). While miR-133a-3p was significantly upregulated at 1 h post the HS training session by FC = 2.25 ($p = 0.003$) (did not reach significance at 1 h post SS training session by FC = 1.84 ($p = 0.003$)), miR-133b-3p was upregulated at 5 min and 1 h following the HS training session (FC 2.2, $p = 0.01$ and FC 2.24, $p = 0.006$, respectively) (Figure 3).

Effect of training specific sessions on serum biomarkers

Levels of muscle damage markers serum creatine kinase (CK) and myoglobin (Mb), and the acute metabolic markers human growth hormone (hGH) and plasma lactate ($[La^-]$) was also analyzed as previously described (Kristoffersen et al., 2018), and at baseline and 5 min, 1 h and 21 h post training session (Supplementary Figure S2). While serum Mb showed peak levels in cyclists 1 h after their first training session (Supplementary Figure S2), plasma $[La^-]$ levels and hGH levels were at its highest 5 min after a SS specific session. The range in CK levels varied largely between individuals and training session (Supplementary Figure S2).

Correlation between miRNAs and biochemical markers following the HS and SS sessions

Post the HS session, no correlation was found between the three miRNAs and biochemical markers. 21 h post the SS session, a negative correlation was however observed between miR-133a-3p and CK ($r = -0.687$, $p = 0.041$) and between miR-133b-3p and $[La^-]$ ($r = -0.711$, $p = .032$).

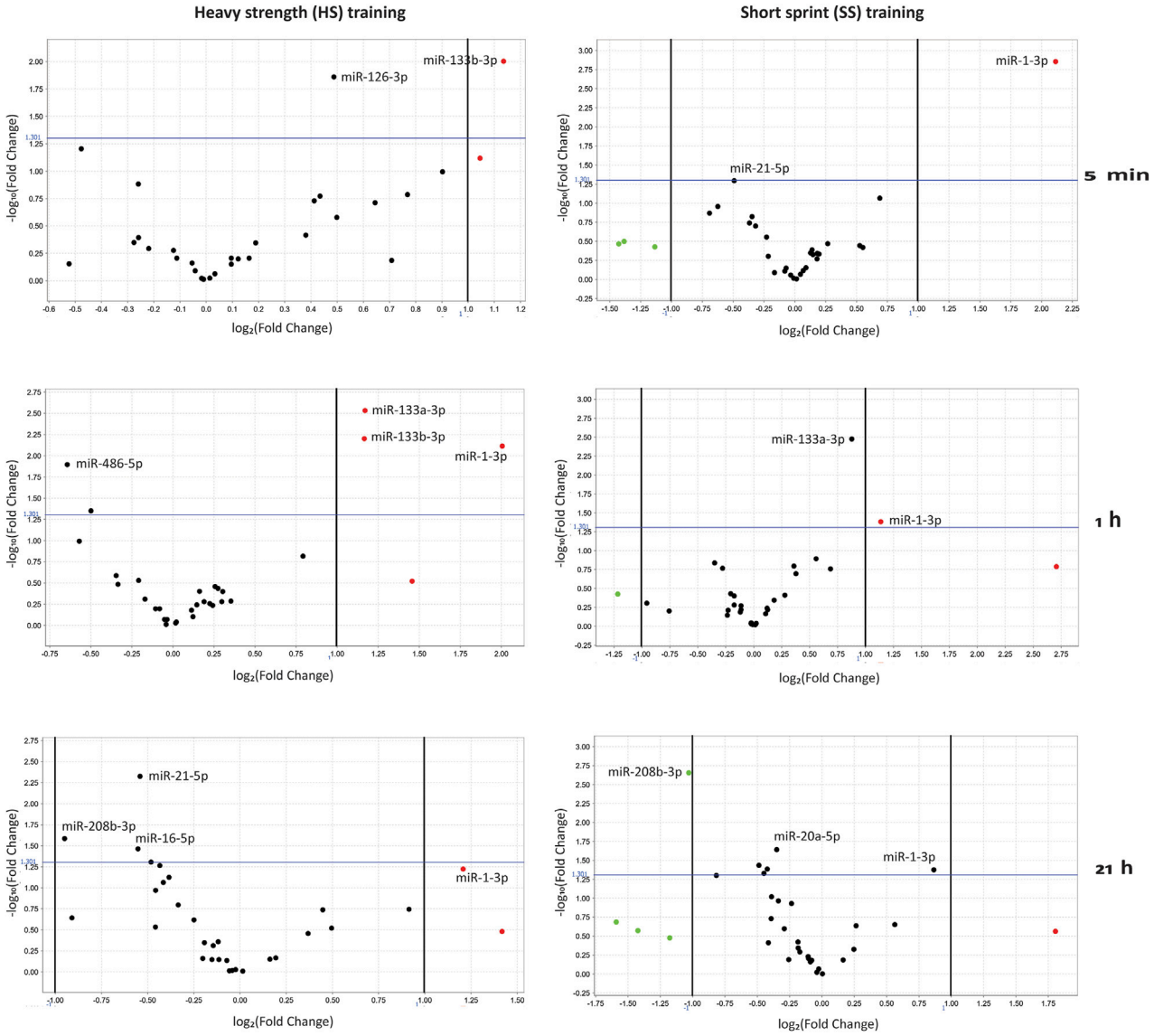


FIGURE 3
Volcano plots of differentially regulated miRNAs at 5 min, 1 h and 21 h post HS (heavy strength) and SS (short sprint) training sessions, normalized to miRNA baseline levels. A change in miRNA expression was considered statistically significant if the Fold Change (FC) was >2.0 and the *p*-value was <0.05.

Discussion

The purpose of our study was to examine the acute response of a single bout of SS and HS training session in elite male cyclists, on the differential expression levels of target c-miRNAs. For this, we collected blood samples from a homogenous group of well-trained male cyclists pre- and post- SS and HS training session, investigating levels of preselected serum c-miRNAs previously associated with altered response to physical exercise by cardiac muscle function, inflammation or osteogenic differentiation, or promoting myogenesis, muscle proliferation or metabolic regulation (Dos Santos et al., 2022). In our pool of c-miRNAs, we found three out of 24 miRNAs to be significantly altered after training sessions. This c-miRNA pool most likely includes both vesicular c-miRNAs (contained in extracellular vesicles such as exosomes, microvesicles or apoptotic bodies) and nonvesicular c-miRNAs (free in circulation as part of RNA-binding protein complexes due to active export systems and/or passive leakage through the plasma membrane following cell damage or death) (Arroyo et al., 2011). Correlation between these three most differentially expressed c-miRNAs and biochemical markers [La^-], Mb, hGH and CK was also assessed.

The acute response of a HS training session most markedly increased the abundance of miR-1-3p, miR-133a-3p and miR-133b-3p at 1 h post HS session. The SS training session most markedly increased the abundance of miR-1-3p already at 5 min post session. These three miRNAs belong to the myomiRs which are preferentially expressed in muscle, with functional roles in for instance promoting myoblast differentiation and regeneration (Horak et al., 2016), but are also detectable in human plasma (Nielsen et al., 2014). They are thought to regulate myogenesis by targeting IGF1/IGF1R and activation of the IGF1/PI3K/AKT

pathway, and with a feed-back loop between IGF1 and AKT/FOXO3, influencing genes encoding myoblast precursors (Domanska-Senderowska et al., 2019).

While has-miR-1-3-p expression levels seems to mimic levels of the acute metabolic marker [La^-] after SS training, and also mimic hGH after second bout of SS training, has-miR-133a-3p on the other hand, seems to follow the muscle damage marker Mb after first bout of HS training (Figure 2; Supplementary Figure S2). The release of Mb is highest after first bout of training during the intervention, while hGH shows highest levels after the second bout, independent of HS>SS or SS>HS. One can speculate if hGH here is affected by a metabolic stress induced during the first bout to increase after a new second bout of training. The opposite is seen for Mb, where muscles after a first bout might be in a kind of contingency for some hours after training.

Resistance training (RT) protocols, differing in the configuration of the acute program variables (e.g., muscle action, loading and volume, exercise pattern and order, rest periods, and repetition velocity and frequency), is known to cause different physiological responses (Bird et al., 2005). Due to this, there is no consensus regarding the optimal way to control such variables. A study investigating the time-course acute responses of 754 human plasma c-miRNAs to different RT protocols (strength endurance, muscular hypertrophy and maximum strength), in regular-exercise students, identified increased abundance of miR-133b 1h post maximum strength training (restored levels at 45 h) (Cui et al., 2017), and in line with our findings 1 h post HS training session. They found, however, no significant change in miR-133a levels. Another study, investigating a subset of miRNAs expressed within skeletal muscle (vastus lateralis) in healthy middle-aged men, in response to strength testing, identified also miR-133a-3p being among five of the 50 most highly expressed miRNAs (Mitchell et al., 2018).

The immediate response of a high-intensity interval exercise trial, in healthy young men, has previously identified significantly increased plasma levels of miR-1, miR-133a and miR-133b (Cui et al., 2016). These findings are somewhat in line with our observed acute response to a single bout of SS training session which significantly increased the abundance of miR-1-3p in blood serum already at 5 min post SS session. Another study investigating the response to a one-off acute sprint interval session in whole blood from healthy men found, however, this physical strain not sufficient to significantly alter miR-1-3p and miR-133a-3p levels (Denham et al., 2018). A study investigating the presence of 800 c-miRNAs in human serum versus plasma samples identified >50% of miRNAs to be uniquely present in either serum or plasma but not both, with a greater diversity of miRNAs in plasma than in serum samples (Foye et al., 2017), and indicating caution when comparing miRNA data generated from different sample types, as well as measurement platforms.

Correlation between c-miRNAs and exercise response and adaptation to a maximal incremental exercise test (VO_2max, HR and maximum aerobic speed), and biochemical markers (CK, [La^-]), in amateur runners, a study by Fernandez-Sanjurjo et al. (2021), identified miRNAs positively or negatively correlating with CK and [La], respectively. None of these did, however, include our miRNAs of interest (miR-1-3p, 133a-3p or 133b-3p). Our analyses showed significant negative correlations between two of our investigated miRNAs, with CK and [La], however extracting true meaningful information from these analyses, performed on such a low number of sample data that was available in this study, is difficult.

Kristoffersen et al. (2018) investigated the presence of DOMS (delayed-onset muscle soreness) post SS and HS training sessions in elite cyclists. Although they reported relatively low DOMS scores, possibly due to the higher fitness levels in the participants, a significantly higher DOMS score (and CK level) was identified 45 h after an HS session (similar levels after 21 h) compared to the SS session. In spite of this, the ability to produce power by the cyclists was found restored back to baseline already 23 h after both training sessions, indicating rapid recovery rate. Comparing this to c-miRNA levels from the same study cohort, miR-1-3p, 133a-3p and 133b-3p had all reached near baseline levels at 21 h post HS session, indicating the relevance of these myomiRs as measures of recovery post HS and SS training, opposing to the participants self-evaluation of DOMS.

There is a lack of a standard protocol for miRNA studies. The literature shows great variation in study design, source of miRNA, extraction methods, study cohort and type of training. In order to control as many variables as possible with importance to c-miRNA response and profiling in our study, the participants were of the same sex, age, had similar sleep pattern, diet, training history and physical activity before the intervention (Kristoffersen et al., 2018), and served as their own control in the intervention. Internal controls for normalization and quantification were implemented in the experimental setup. There are however several limitations to our study. Firstly, our study was based on a low number of participants in the intervention. Additional participants would most likely have provided better statistics. Secondly, we assessed miRNAs in serum samples; a sample type reported to contain lower miRNA diversity than plasma samples, possibly as a result of the coagulation process or cellular remnants (Sapp et al., 2017). Another preanalytical variable is hemolysis which can increase some miRNAs and be a confounder in these studies (McDonald et al., 2011). A hemolyzation quality control should ideally be incorporated, as well as comparing miRNA profiling in both serum and plasma. Thirdly, and due to economy, the methodology available limited the analysis to a group of pre-selected miRNAs reported to be altered after diverse acute exercises, and restrictions were imposed to the number of time-points allowed during the intervention. Future investigations might prefer global miRNA analysis, to identify novel c-miRNAs associated with training adaption and recovery or considering next-generation sequencing (NGS) as gold standard for miRNA-profiling due to greater detection sensitivity and accuracy in differential expression analysis, compared to quantitative real-time PCR. Also, longitudinal samples, collected at multiple time-points following several training sessions and longer period (months rather than hours), are likely to provide more relevant insights into the recovery and the role miRNAs might play here over time.

In conclusion our findings demonstrate that heavy strength and/or short sprint training in well-trained cyclists can increase the circulating levels of some of the miRNAs associated with muscle development. Although the roles of circulating small non-coding miRNAs are yet to be fully elucidated, our data indicate the relevance of myomiRs miR-1-3p, 133a-3p and 133b-3p as measures of acute response and recovery status.

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 humans were approved by The Norwegian Data Protection Authority. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

AR: Conceptualization, Data curation, Methodology, Supervision, Validation, Writing–original draft, Writing–review and editing, Formal Analysis, Funding acquisition, Project administration. KR: Conceptualization, Data curation, Investigation, Methodology, Validation, Writing–original draft, Formal Analysis, Funding acquisition. EE: Conceptualization, Methodology, Validation, Writing–original draft, Data curation. GS: Data curation, Investigation, Methodology, Software, Validation, Writing–review and editing, Funding acquisition. GP: Conceptualization, Data curation, Methodology, Validation, Writing–review and editing. HG: Conceptualization, Data curation, Methodology, Validation, Writing–review and editing. MK: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Validation, Writing–review and editing, Formal Analysis. LB: Data curation, Formal Analysis, Investigation, Software, Validation, Writing–original draft, Writing–review and editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. This study was supported by grant from Olympiatoppen and from the Western Norway University of Applied Sciences.

Acknowledgments

We are very grateful to Prof. Dr. med Karl-Henning Kalland (Centre for Cancer Biomarkers CCBIO, Department of Clinical Science, University of Bergen) for lending us laboratory space and the use of his Applied Biosystems 7900HT Real-Time PCR machine to run the TLDA-cards. We are also grateful to Paal Henning Borge (Western Norway University of Applied Sciences) for technical assistance in the miRNA analysis preparations.

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.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2024.1365357/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Unsupervised hierarchical clustering analysis of miRNAs in serum. The heat map illustrates the result of the one-way hierarchical clustering of 24 miRNAs, including endogenous- and spike-in controls. Samples derived from 9 cyclists were analyzed at all timepoints pre-/post SS and HS training sessions, using the TLDA assay. Each row represents one miRNA and each column represents one sample. Similarities between samples were analyzed by Euclidean Distance, complete linkage method, and map type global (ΔCT), using the ExpressionSuite software. * denotes spike-in controls; ** denotes endogenous controls; CTL indicates mandatory array card control (cel-miR-39-3p). The color scale shown to the left illustrates the relative expression levels of miRNA across all samples: red color represents expression level above the mean, blue color represents expression level lower than the mean (confidence level 95%). Cyclists and training session time points (IDs) are shown horizontally below each figure. Clustering was performed on **(A)** all miRNAs and **(B)** miRNAs that have significant different expression level with $p < 0.05$.

SUPPLEMENTARY FIGURE S2
Dot-plots of biochemical marker levels in serum following cyclists' training sessions. Changes in levels of creatine kinase (CK) **(A, B)**, plasma lactate (La^-) **(C, D)**, serum myoglobin (Mb) **(E, F)**, and human growth hormone (hGH) **(G, H)**, following a heavy strength training session and thereafter a short sprint session (HS>SS) **(A, C, E, G)**, or following a short sprint training session and thereafter a heavy strength session (SS>HS) **(B, D, F, H)**. Biochemical marker levels were measured at baseline and post-exercise (5 min, 1 h, 21 h and 45 h/baseline) in nine well-trained cyclists. Mean is shown as a horizontal line.

References

Arroyo, J. D., Chevillet, J. R., Kroh, E. M., Ruf, I. K., Pritchard, C. C., Gibson, D. F., et al. (2011). Argonaute2 complexes carry a population of circulating microRNAs independent of vesicles in human plasma. *Proc. Natl. Acad. Sci. U. S. A.* 108 (12), 5003–5008. doi:10.1073/pnas.1019055108

Batterham, A. M., and Hopkins, W. G. (2006). Making meaningful inferences about magnitudes. *Int. J. Sports Physiol. Perform.* 1 (1), 50–57. doi:10.1123/ijspp.1.1.50

Bertucci, W., Taiar, R., and Grappe, F. (2005). Differences between sprint tests under laboratory and actual cycling conditions. *J. Sports Med. Phys. Fit.* 45 (3), 277–283.

Bird, S. P., Tarpenning, K. M., and Marino, F. E. (2005). Designing resistance training programmes to enhance muscular fitness: a review of the acute programme variables. *Sports Med.* 35 (10), 841–851. doi:10.2165/00007256-200535100-00002

Camera, D. M., Smiles, W. J., and Hawley, J. A. (2016). Exercise-induced skeletal muscle signaling pathways and human athletic performance. *Free Radic. Biol. Med.* 98, 131–143. doi:10.1016/j.freeradbiomed.2016.02.007

Cohen, J. (1988). Statistical power analysis for the behavioural sciences. 2nd edn Hillsdale, N. J.: Laurence Earlbaum Associates [Google Scholar: https://books.google.no/books?hl=no&lr=&id=rEe0BQAAQBAJ&oi=fnd&pg=PP1&ots=sxTZMtOUsc&sig=ocF7ziDNDaWuCcaXF9afSNsxOc&redir_esc=y#v=onepage&q&f=false.

Cui, S., Sun, B., Yin, X., Guo, X., Chao, D., Zhang, C., et al. (2017). Time-course responses of circulating microRNAs to three resistance training protocols in healthy young men. *Sci. Rep.* 7 (1), 2203. doi:10.1038/s41598-017-02294-y

Cui, S. F., Wang, C., Yin, X., Tian, D., Lu, Q. J., Zhang, C. Y., et al. (2016). Similar responses of circulating MicroRNAs to acute high-intensity interval exercise and vigorous-intensity continuous exercise. *Front. Physiol.* 7, 102. doi:10.3389/fphys.2016.00102

Del Vecchio, L., Stanton, R., Reaburn, P., Macgregor, C., Meerkin, J., Villegas, J., et al. (2019). Effects of combined strength and sprint training on lean mass, strength, power, and sprint performance in masters road cyclists. *J. Strength Cond. Res.* 33 (1), 66–79. doi:10.1519/JSC.0000000000001960

Denham, J., Gray, A., Scott-Hamilton, J., and Hagstrom, A. D. (2018). Sprint interval training decreases circulating MicroRNAs important for muscle development. *Int. J. Sports Med.* 39 (1), 67–72. doi:10.1055/s-0043-120763

Domanska-Senderowska, D., Laguette, M. N., Jegier, A., Cieszczyk, P., September, A. V., and Brzezianska-Lasota, E. (2019). MicroRNA profile and adaptive response to exercise training: a review. *Int. J. Sports Med.* 40 (4), 227–235. doi:10.1055/a-0824-4813

Dos Santos, J. A. C., Veras, A. S. C., Batista, V. R. G., Tavares, M. E. A., Correia, R. R., Suggett, C. B., et al. (2022). Physical exercise and the functions of microRNAs. *Life Sci.* 304, 120723. doi:10.1016/j.lfs.2022.120723

Egan, B., and Zierath, J. R. (2013). Exercise metabolism and the molecular regulation of skeletal muscle adaptation. *Cell Metab.* 17 (2), 162–184. doi:10.1016/j.cmet.2012.12.012

Fernandez-Sanjurjo, M., Diaz-Martinez, A. E., Diez-Robles, S., Gonzalez-Gonzalez, F., de Gonzalo-Calvo, D., Rabadan, M., et al. (2021). Circulating MicroRNA profiling reveals specific subsignatures in response to a maximal incremental exercise test. *J. Strength Cond. Res.* 35 (2), 287–291. doi:10.1519/JSC.0000000000003930

Foye, C., Yan, I. K., David, W., Shukla, N., Habboush, Y., Chase, L., et al. (2017). Comparison of miRNA quantitation by Nanostring in serum and plasma samples. *PLoS One* 12 (12), e0189165. doi:10.1371/journal.pone.0189165

Gundersen, K. (2011). Excitation-transcription coupling in skeletal muscle: the molecular pathways of exercise. *Biol. Rev. Camb Philos. Soc.* 86 (3), 564–600. doi:10.1111/j.1469-185X.2010.00161.x

Hopker, J., Myers, S., Jobson, S. A., Bruce, W., and Passfield, L. (2010). Validity and reliability of the Wattbike cycle ergometer. *Int. J. Sports Med.* 31 (10), 731–736. doi:10.1055/s-0030-1261968

Horak, M., Novak, J., and Bienertova-Vasku, J. (2016). Muscle-specific microRNAs in skeletal muscle development. *Dev. Biol.* 410 (1), 1–13. doi:10.1016/j.ydbio.2015.12.013

Horak, M., Zlamal, F., Iliev, R., Kucera, J., Cacek, J., Svobodova, L., et al. (2018). Exercise-induced circulating microRNA changes in athletes in various training scenarios. *PLoS One* 13 (1), e0191060. doi:10.1371/journal.pone.0191060

Hughes, D. C., Ellefsen, S., and Baar, K. (2018). Adaptations to endurance and strength training. *Cold Spring Harb. Perspect. Med.* 8 (6), a029769. doi:10.1101/cshperspect.a029769

Jeukendrup, A. E., Craig, N. P., and Hawley, J. A. (2000). The bioenergetics of world class cycling. *J. Sci. Med. Sport* 3 (4), 414–433. doi:10.1016/s1440-2440(00)80008-0

Kosaka, N., Yoshioka, Y., Hagiwara, K., Tominaga, N., Katsuda, T., and Ochiya, T. (2013). Trash or Treasure: extracellular microRNAs and cell-to-cell communication. *Front. Genet.* 4, 173. doi:10.3389/fgene.2013.00173

Kraemer, W. J., Vescovi, J. D., Volek, J. S., Nindl, B. C., Newton, R. U., Patton, J. F., et al. (2004). Effects of concurrent resistance and aerobic training on load-bearing performance and the Army physical fitness test. *Mil. Med.* 169 (12), 994–999. doi:10.7205/milmed.169.12.994

Kristoffersen, M., Sandbakk, O., Ronnestad, B. R., and Gundersen, H. (2019). Comparison of short-sprint and heavy strength training on cycling performance. *Front. Physiol.* 10, 1132. doi:10.3389/fphys.2019.01132

Kristoffersen, M., Sandbakk, O., Tonnessen, E., Svendsen, I., Paulsen, G., Ersvaer, E., et al. (2018). Power production and biochemical markers of metabolic stress and muscle damage following a single bout of short-sprint and heavy strength exercise in well-trained cyclists. *Front. Physiol.* 9, 155. doi:10.3389/fphys.2018.00155

Krol, J., Loedige, I., and Filipowicz, W. (2010). The widespread regulation of microRNA biogenesis, function and decay. *Nat. Rev. Genet.* 11 (9), 597–610. doi:10.1038/nrg2843

Li, L., Zhu, D., Huang, L., Zhang, J., Bian, Z., Chen, X., et al. (2012). Argonaute 2 complexes selectively protect the circulating microRNAs in cell-secreted microvesicles. *PLoS One* 7 (10), e46957. doi:10.1371/journal.pone.0046957

Li, Y., Yao, M., Zhou, Q., Cheng, Y., Che, L., Xu, J., et al. (2018). Dynamic regulation of circulating microRNAs during acute exercise and long-term exercise training in basketball athletes. *Front. Physiol.* 9, 282. doi:10.3389/fphys.2018.00282

Martin, J. C. (2007). Muscle power: the interaction of cycle frequency and shortening velocity. *Exerc Sport Sci. Rev.* 35 (2), 74–81. doi:10.1097/jes.0b013e31803eb0a0

Massart, J., Sjogren, R. J. O., Egan, B., Garde, C., Lindgren, M., Gu, W., et al. (2021). Endurance exercise training-responsive miR-19b-3p improves skeletal muscle glucose metabolism. *Nat. Commun.* 12 (1), 5948. doi:10.1038/s41467-021-26095-0

McDonald , J. A., Milosevic, D., Reddi, H. V., Grebe, S. K., and Algeciras-Schimnich, A. (2011). Analysis of circulating microRNA: preanalytical and analytical challenges. *Clinical chem.* 57 (6), 833–840. doi:10.1373/clinchem.2010.157198

Menaspa, P., Quod, M., Martin, D. T., Peiffer, J. J., and Abbiss, C. R. (2015). Physical demands of sprinting in professional road cycling. *Int. J. Sports Med.* 36 (13), 1058–1062. doi:10.1055/s-0035-1554697

Mitchell, C. J., D'Souza, R. F., Schierding, W., Zeng, N., Ramzan, F., O'Sullivan, J. M., et al. (2018). Identification of human skeletal muscle miRNA related to strength by high-throughput sequencing. *Physiol. Genomics* 50 (6), 416–424. doi:10.1152/physiolgenomics.00112.2017

Mitchell, P. S., Parkin, R. K., Kroh, E. M., Fritz, B. R., Wyman, S. K., Pogosova-Agadjanyan, E. L., et al. (2008). Circulating microRNAs as stable blood-based markers for cancer detection. *Proc. Natl. Acad. Sci. U. S. A.* 105 (30), 10513–10518. doi:10.1073/pnas.0804549105

Nielsen, S., Akerstrom, T., Rinnov, A., Yfanti, C., Scheele, C., Pedersen, B. K., et al. (2014). The miRNA plasma signature in response to acute aerobic exercise and endurance training. *PLoS One* 9 (2), e87308. doi:10.1371/journal.pone.0087308

Rayner, K. J., and Hennessy, E. J. (2013). Extracellular communication via microRNA: lipid particles have a new message. *J. Lipid Res.* 54 (5), 1174–1181. doi:10.1194/jlr.R034991

Ronnestad, B. R., Hansen, E. A., and Raastad, T. (2010). Effect of heavy strength training on thigh muscle cross-sectional area, performance determinants, and performance in well-trained cyclists. *Eur. J. Appl. Physiol.* 108 (5), 965–975. doi:10.1007/s00421-009-1307-z

Sapp, R. M., Shill, D. D., Dash, C., Hicks, J. C., Adams-Campbell, L. L., and Hagberg, J. M. (2019). Circulating microRNAs and endothelial cell migration rate are associated with metabolic syndrome and fitness level in postmenopausal African American women. *Physiol. Rep.* 7 (14), e14173. doi:10.14814/phy2.14173

Sawada, S., Kon, M., Wada, S., Ushida, T., Suzuki, K., and Akimoto, T. (2013). Profiling of circulating microRNAs after a bout of acute resistance exercise in humans. *PLoS One* 8 (7), e70823. doi:10.1371/journal.pone.0070823

Shah, M. Y., and Calin, G. A. (2013). The mix of two worlds: non-coding RNAs and hormones. *Nucleic Acid. Ther.* 23 (1), 2–8. doi:10.1089/nat.2012.0375

Skovereng, K., Sylta, O., Tonnessen, E., Hammarstrom, D., Danielsen, J., Seiler, S., et al. (2018). Effects of initial performance, gross efficiency and O (2peak) characteristics on subsequent adaptations to endurance training in competitive cyclists. *Front. Physiol.* 9, 713. doi:10.3389/fphys.2018.00713

Tsui, N. B., Ng, E. K., and Lo, Y. M. (2002). Stability of endogenous and added RNA in blood specimens, serum, and plasma. *Clin. Chem.* 48 (10), 1647–1653. doi:10.1093/clinchem/48.10.1647

Uhlemann, M., Mobius-Winkler, S., Fikenzer, S., Adam, J., Redlich, M., Mohlenkamp, S., et al. (2014). Circulating microRNA-126 increases after different forms of endurance exercise in healthy adults. *Eur. J. Prev. Cardiol.* 21 (4), 484–491. doi:10.1177/2047487312467902

Valadi, H., Ekstrom, K., Bossios, A., Sjostrand, M., Lee, J. J., and Lotvall, J. O. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.* 9 (6), 654–659. doi:10.1038/ncb1596

Vickers, K. C., Palmisano, B. T., Shoucri, B. M., Shamburek, R. D., and Remaley, A. T. (2011). MicroRNAs are transported in plasma and delivered to recipient cells by high-density lipoproteins. *Nat. Cell Biol.* 13 (4), 423–433. doi:10.1038/ncb2210

Vikmoen, O., Ellefsen, S., Troen, O., Hollan, I., Hanestadhaugen, M., Raastad, T., et al. (2016). Strength training improves cycling performance, fractional utilization of VO2max and cycling economy in female cyclists. *Scand. J. Med. Sci. Sports* 26 (4), 384–396. doi:10.1111/sms.12468

Zernecke, A., Bidzhekov, K., Noels, H., Shagdarsuren, E., Gan, L., Denecke, B., et al. (2009). Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Sci. Signal* 2 (100), ra81. doi:10.1126/scisignal.2000610

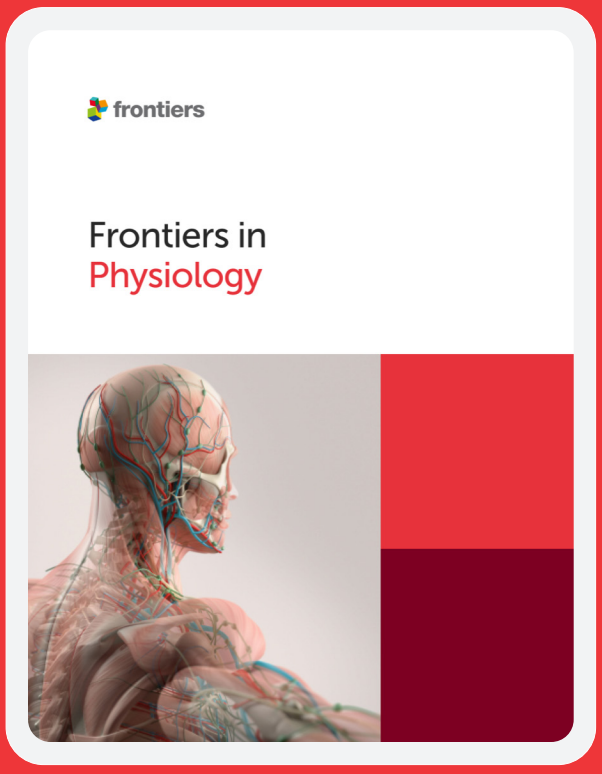
Frontiers in Physiology

Understanding how an organism's components work together to maintain a healthy state

The second most-cited physiology journal, promoting a multidisciplinary approach to the physiology of living systems - from the subcellular and molecular domains to the intact organism and its interaction with the environment.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact