From the Institute of Sport Science

in the Faculty 06 Psychology and Sports Science

Professorship for Exercise Physiology and Sports Therapy

JUSTUS-LIEBIG-
UNIVERSITÄT
GIESSEN

DISSERTATION

Respiratory Adaptations of Immune Cells:

Metabolic Links Between Exercise,

Cardiorespiratory Fitness and Immunity

for the Award of the Doctoral Degree

Doctor rerum naturalium (Dr. rer. nat.)

at the Justus Liebig University of Giessen

submitted by

Kristina Marie Gebhardt

Giessen, 2026

Acknowledgements

I would like to sincerely thank my supervisor, Prof. Dr. Karsten Krüger, for his guidance, support, and trust throughout this work. I also thank Prof. Dr. Natascha Sommer for her critical insights and for providing me with the opportunity to learn and conduct laboratory work at the Excellence Cluster Cardio-Pulmonary Institute (CPI).

My sincere appreciation also goes to my co-authors and colleagues at the Department of Exercise Physiology and Sports Therapy, in particular Dr. Christopher Weyh, whose collaboration and insightful discussions greatly enriched this research. I am also grateful to everybody who contributed to the studies and enriched this work through their ideas and support.

Finally, I am deeply grateful to my family and friends for their encouragement, patience, and constant support along this journey.

Board of Examiners

First Reviewer:

Prof. Dr. Karsten Krüger
FB06 – Psychology and Sports Science
Department of Exercise Physiology and Sports Therapy
Justus Liebig University of Giessen
Kugelberg 62
D-35394 Giessen
Germany
Karsten.Krueger@sport.uni-giessen.de

Second Reviewer:

Prof. Dr. Christoph Rummel
FB10 – Veterinary Medicine
Institute of Veterinary Physiology and Biochemistry
Justus Liebig University of Giessen
Frankfurter Strasse 100
D-35392 Giessen
Germany
Christoph.D.Rummel@vetmed.uni-giessen.de

President of Justus Liebig University of Giessen: Prof. Dr. Katharina Lorenz
Dean of Faculty 06: Prof. Dr. Christiane Hermann

Preface

The present work was prepared as a cumulative dissertation in accordance with the regulations of the doctoral degree guidelines of the Faculty 06 Psychology and Sports Science at Justus Liebig University Giessen. The selected original publications have been included in order of their content to provide a detailed presentation of the methods and materials used, the experimental data, and a comprehensive discussion of the respective results.

Publications Included in This Thesis

This cumulative dissertation is based on the following published works:

Publication 1:

Gebhardt K, Hebecker A, Honekamp C, Nolte S, Barthkuhn M, Wilhelm J, Klatt S, Weyh C, Sommer N, and Krüger K (2024). Respiratory and Metabolic Responses of CD4+ T Cells to Acute Exercise and Their Association with Cardiorespiratory Fitness. Medicine & Science in Sports & Exercise, 56(10), 1882-1892.
DOI: https://doi.org/10.1249/MSS.0000000000003483
Supplementary data and materials were published online as an appendix to the article at:
http://links.lww.com/MSS/D42

Publication 2:

Gebhardt K, Hebecker A, Sommer N, Ringseis R, Eder K, Huber M, Raifer H, Krüger K, and Weyh C (2025). PBMCs Mitochondrial Respiration and Its Relation to Immunity, Fitness, and Metabolic Risk in the Healthy Elderly. Journal of Cellular Physiology, 240(9), e70096.
DOI: https://doi.org/10.1002/jcp.70096
Supplementary data and materials were published online as an appendix to the article at:
https://onlinelibrary.wiley.com/doi/10.1002/jcp.70096

Publication 3:

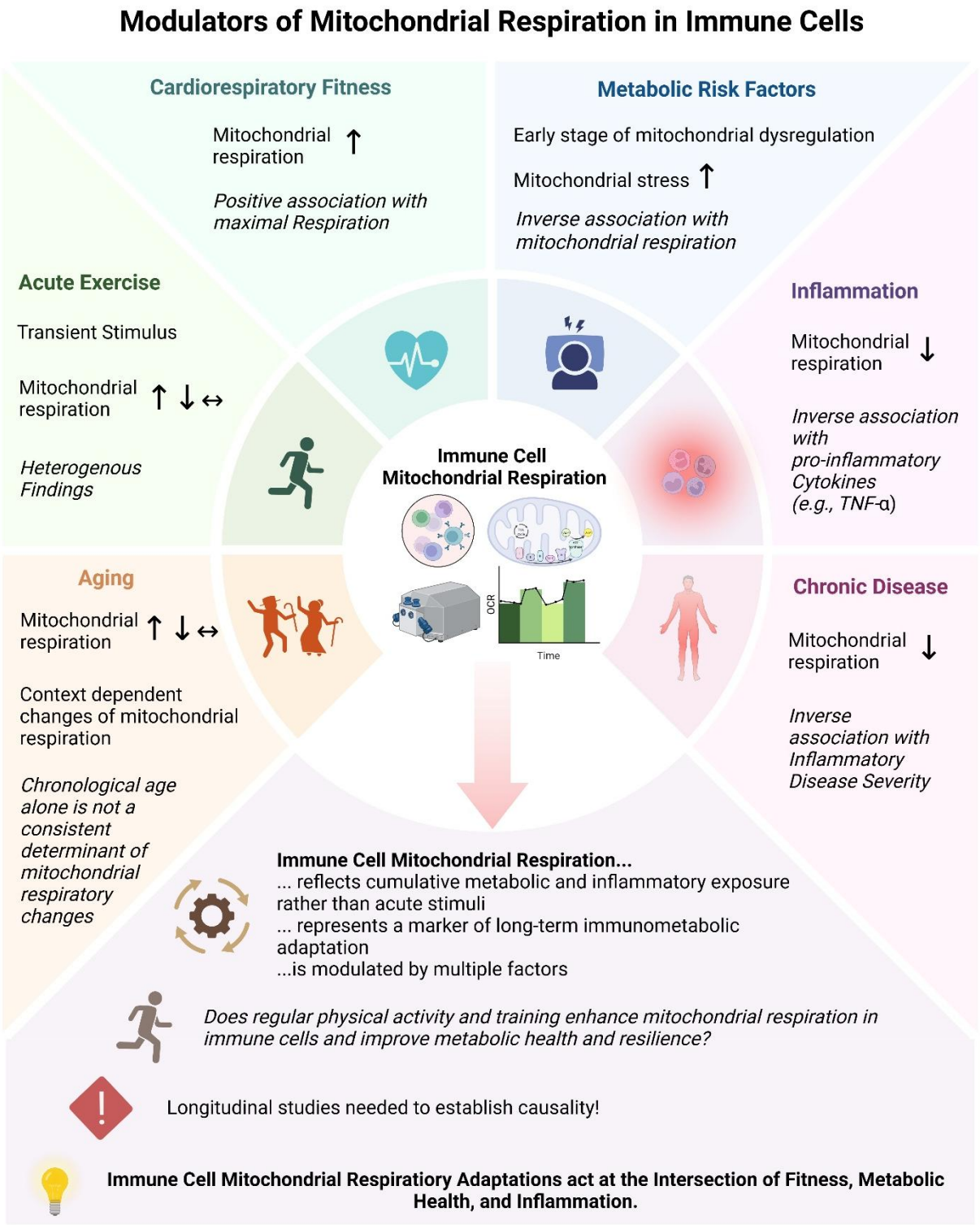
Gebhardt K and Krüger K (2024). Effects of Cardiorespiratory Fitness on Immune Cell Mitochondrial Metabolism in Health and Disease. Dtsch Z Sportmed. 2024; 75:123-131.
DOI: https://doi.org/10.5960/dzsm.2024.587
Supplementary data and materials were published online as an appendix to the article at:
https://www.germanjournalsportsmedicine.com/archive/archive-2024/issue-4/effects-of-cardiorespiratory-fitness-on-immune-cell-mitochondrial-metabolism-in-health-and-disease/

List of Abbreviations

ADP	Adenosine Diphosphate
AMPK	AMP-Activated Protein Kinase
ATP	Adenosine Triphosphate
c-Myc	Cellular Myelocytomatosis Oncogene
CM	Central Memory T Cell
CRF	Cardiorespiratory Fitness
DR	Downhill Running
EM	Effector Memory T Cell
EMRA	Terminal Effector Memory T Cell
ETC	Electron Transport Capacity
ETS	Electron Transport System
FAO	Fatty Acid Oxidation
FCCP	Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone
FOXP3	Forkhead box P3
GLUT	Glucose Transporter
HbA1c	Hemoglobin A1C
HIF-1α	Hypoxia-inducible Factor-1 alpha
HRR	High-Resolution Respirometry
ICAM-1	Intercellular Adhesion Molecule-1
IKK	Inhibitor of Nuclear Factor B
IMM	Inner Mitochondrial Membrane
LDL	Low-density Lipoprotein
LEA	Low Energy Availability
LR	Level Running
MAPK	Mitogen-activated Protein Kinase
mTOR	Mechanistic Target of Rapamycin
mtDNA	Mitochondrial DNA
mTreg	Memory regulatory T Cell
NF-κB	Nuclear factor kappa B
NK Cell	Natural Killer Cell
NLRP3	NOD-like Receptor Family Pyrin Domain Containing 3
OXPHOS	Oxidative Phosphorylation
PA	Physical Activity

PBMCs	Peripheral Blood Mononuclear Cells
PGC1-α	Peroxisome Proliferator-activated Receptor Gamma Coactivator 1-alpha
PI3K-Akt	Phosphoinositide 3-kinase-Akt Signaling Pathway
PPAR	Peroxisome Proliferator-activated Receptor
PTM	Post-translational Modification
REDs	Relative Energy Deficiency in Sport
ROS	Reactive Oxygen Species
rTreg	Resting Regulatory T Cell
SCENITH	Single Cell Energetic Interrogation of Translational Health
SCM	Stem cell-like Memory T Cell
STAT3	Signal Transducer and Activator of Transcription 3
SUIT	Substrate-uncoupler-inhibitor Titration
TCA Cycle	Tricarboxylic Acid Cycle
Teff	Effector T Cell
Tfh	T Follicular Helper Cell
TLR-4	Toll-like Receptor 4
TNF-α	Tumor Necrosis Factor Alpha
Tmem	Memory T Cell
Treg	Regulatory T Cell
VEGF	Vascular endothelial growth factor
VO_2max	Maximal Oxygen Uptake

Graphical Abstract



Created in BioRender

Abstract

Background: This dissertation investigates how acute exercise, cardiorespiratory fitness (CRF), metabolic risk factors, inflammation, and aging influence mitochondrial respiration (MR) in human peripheral blood mononuclear cells (PBMCs) and $CD4^+$ T cells. The aim was to examine whether MR reflects adaptive immunometabolic responses across different contexts.
Methods: To examine modulators of MR in immune cells, three complementary studies were conducted. The first study investigated acute exercise-induced immunometabolic responses in healthy young adults (n = 15). After VO_2max testing, participants performed two randomized treadmill sessions, consisting of 45-minutes of downhill running (12% decline) and level running (1% incline) at 70% of VO_2max. Blood samples were collected before, immediately after, 3 hours after, and 24 hours after exercise. Freshly isolated $CD4^+$ T cells were incubated in autologous serum obtained before and three hours after each exercise. MR was assessed by measuring oxygen consumption using high-resolution respirometry (HRR) with Oroboros O2k Oxygraph, complemented by transcriptomic, metabolomic, cytokine, and immune cell subset analyses. The second study examined MR of PBMCs in healthy older adults (n = 16, >55 years) under resting conditions. Following fasting blood sampling and CRF assessment via VO_2max testing, PBMC MR was measured using HRR with Oroboros O2k Oxygraph. Associations with immune cell subsets, cytokines, metabolic markers, and lifestyle-related factors were analyzed. The third study comprised a narrative review of 20 studies employing HRR (Oroboros O2k Oxygraph or Seahorse XF Analyzer) to assess MR in PBMCs and their subtypes, as well as in platelets and neutrophils. The review examined the effects of acute exercise, training, and CRF across healthy and diseased populations to frame the current knowledge about MR in the field of exercise immunology.
Results: The MR of PBMCs and $CD4^+$ T cells is modulated through different factors. Acute endurance exercise induced a transient systemic inflammatory response but resulted in minor changes in MR of $CD4^+$ T cells. In contrast, higher CRF was associated with enhanced maximal MR, accompanied by increased expression of genes involved in oxidative phosphorylation and a metabolite profile indicative of improved metabolic efficiency. In healthy older adults, higher basal MR was associated with lower pro-inflammatory cytokine levels such as TNF-α and IL-17. Higher maximal MR was linked to greater proportions of $CD4^+$, $CD8^+$, and naïve $CD4^+$ T cells, and was further associated with STAT3, VEGF, and ICAM-1. The narrative review supported these findings, demonstrating that higher CRF and long-term training are frequently associated with improved MR in immune cells, whereas chronic diseases and systemic inflammation are characterized by impaired mitochondrial respiratory function.
Conclusion: Collectively, this dissertation demonstrates that MR of immune cells represents a long-term marker of immunometabolic adaptation. MR of immune cells, particularly PBMCs and $CD4^+$ T cells, appears to be modulated by multiple factors, including exercise, CRF, metabolic risk, inflammatory status, immune cell composition, and diseases. These findings place immune cell MR at the intersection of fitness, metabolic health, and inflammation. Future longitudinal studies should investigate whether long-term exercise interventions can enhance MR and thereby improve immunometabolic resilience, to examine causal relationships.

Zusammenfassung

Hintergrund: Diese Dissertation untersucht, wie akute körperliche Belastung, kardiorespiratorische Fitness (CRF), Alterung und metabolische Risikofaktoren die mitochondriale Respiration (MR) in humanen peripheren mononukleären Blutzellen (PBMCs) und $CD4^+$ T-Zellen beeinflussen. Ziel war es, zu untersuchen, ob die MR immunmetabolische Adaptationen in unterschiedlichen Kontexten widerspiegelt.
Methoden: Drei komplementäre Studien wurden durchgeführt. Die erste Studie untersuchte die Effekte akuter Belastung bei gesunden jungen Erwachsenen (n = 15). Nach Testung der maximalen Sauerstoffaufnahme (VO_2max) wurden ein Bergablauf (12% Gefälle) und ein Geradeauslauf (1% Steigung) für 45 Minuten bei 70% der VO_2max absolviert. Blutproben wurden vor, direkt nach, 3 und 24 Stunden nach der Belastung entnommen. Frisch isolierte $CD4^+$ T-Zellen wurden in autologem Serum von vor und 3 Stunden nach Belastung inkubiert. Die MR wurde mittels hochauflösender Respirometrie (HRR; Oroboros O2k) bestimmt und durch transkriptomische, metabolomische, zytokinbasierte sowie Subpopulations-Analysen ergänzt. Die zweite Studie untersuchte die MR von PBMCs bei gesunden Älteren (n = 16; Alter > 55 Jahre). Nach einer Nüchtern-Blutentnahme und einem VO_2max-Test wurde die MR der PBMCs mittels HRR gemessen. Assoziationen mit Subpopulationen, Zytokinen, metabolischen Markern und Lebensstilfaktoren wurden analysiert. Die dritte Studie umfasste eine Übersichtsarbeit mit 20 Studien, in denen mittels HRR (Oroboros O2k/ Seahorse XF Analyzer) die MR verschiedener Immunzellen untersucht wurde, um Assoziationen zu akuter Belastung, Training, CRF sowie unterschiedlichen Gesundheits- und Krankheitszuständen zu analysieren und die Studienlage in der Sportimmunologie zusammenzufassen.
Ergebnisse: Die MR von PBMCs und $CD4^+$ T-Zellen wurde durch verschiedene Einflussfaktoren moduliert. Akute Belastung führte zu einer vorübergehenden systemischen Entzündungsreaktion, bewirkte jedoch nur geringe Veränderungen der MR in $CD4^+$ T-Zellen. Eine höhere CRF war mit einer gesteigerten maximalen MR assoziiert, begleitet von einer erhöhten Expression von Genen der oxidativen Phosphorylierung sowie einem Metabolit-Profil, das auf eine verbesserte metabolische Effizienz hinweist. Bei gesunden Älteren war eine höhere basale MR mit niedrigeren Konzentrationen proinflammatorischer Zytokine (TNF-α, IL-17) verbunden. Die maximale MR war mit einem größeren Anteil an $CD4^+$-, $CD8^+$- und naiven $CD4^+$ T-Zellen verbunden und zeigte Assoziationen mit STAT3, VEGF und ICAM-1. Das Review bestätigte, dass eine höhere CRF mit einer höheren MR sowie Erkrankungen und systemische Entzündungen mit einer niedrigeren MR von Immunzellen assoziiert sind.
Fazit: Zusammenfassend zeigt diese Dissertation, dass die MR von Immunzellen einen langfristigen Marker immunmetabolischer Anpassungsprozesse darstellt. Die MR wird durch mehrere Faktoren wie körperliche Aktivität, CRF, metabolische Risikofaktoren und Gesundheitsstatus moduliert. Damit steht die MR von Immunzellen an der Schnittstelle zwischen Fitness, metabolischer Gesundheit und Entzündungsprozessen. Zukünftige Studien sollten untersuchen, ob langfristige Trainingsinterventionen die MR verbessern und dadurch die immunmetabolische Resilienz steigern können. Dabei sollen die kausalen Zusammenhänge gezielt erforscht werden.

Contents

Acknowledgements I
Board of Examiners II
Preface III
Publications Included in This Thesis IV
List of Abbreviations V
Graphical Abstract VII
Abstract VIII
Zusammenfassung IX
Contents X
1 General Introduction 1
2 Aim of This Dissertation Thesis 3
3 Theoretical Background 4
3.1 Functional Characteristics and Immunometabolism of PBMCs with a Focus on CD4⁺ T Cells 4
3.2 Mitochondrial Function and Respiration in PBMCs and CD4⁺ T Cells 6
3.2.1 Mitochondrial Metabolism and the Electron Transport System 6
3.2.2 Assessment of Mitochondrial Respiratory States 7
3.2.3 Mechanisms of Mitochondrial Respiratory Function in Health & Disease 8
3.3 Insights into Potential Modulators of Mitochondrial Respiratory Function 9
3.3.1 Exercise and Cardiorespiratory Fitness 9
3.3.2 Immunometabolism in the Elderly 11
3.3.3 Inflammation and Chronic Disease 12
4 Synopsis 13
4.1 Conceptual Framework of this Dissertation Thesis 13
4.2 Overview of the Included Studies 13
4.2.1 Study Section I (Gebhardt et al., 2024) 13
4.2.2 Study Section II (Gebhardt et al., 2025) 14
4.2.3 Study Section III (Gebhardt & Krüger, 2024) 14
4.3 Integrated Interpretation of Findings 14
4.3.1 Acute Exercise and CRF as Modulators of Mitochondrial Respiratory Adaptation in CD4⁺ T cells 14

4.3.2 Mitochondrial Respiration of PBMCs and Metabolic Markers in Healthy Aged Individuals 15

4.3.3 Mitochondrial Respiration of PBMCs and Subtypes as an Integrative Marker of Acute Exercise, CRF, and Inflammation in Health and Disease 15

5 Publications 16

5.1 Respiratory and Metabolic Responses of $CD4^+$ T cells to Acute Exercise and Their Association with Cardiorespiratory Fitness 16

5.2 PBMCs Mitochondrial Respiration and Its Relation to Immunity, Fitness, and Metabolic Risk in the Healthy Elderly 17

5.3 Effects of Cardiorespiratory Fitness on Immune Cell Mitochondrial Metabolism in Health and Disease 30

6 Discussion 39

7 Potential Relevance and Future Perspectives 46

8 Conclusion 48

References 49

Formal Declaration 58

Appendix 59

1 General Introduction

As one of the body's largest organ systems, the immune system plays a central role in maintaining health by defending against pathogens, regulating inflammation, and supporting tissue repair [1]. These functions critically depend on the close interplay between immune function and cellular metabolism, termed immunometabolism, with immune cells dynamically adapting their metabolic programs depending on their activation state and functional demands [2, 3]. Central to these processes are mitochondria, which govern the functionality, adaptability, and resilience of cellular energy metabolism, enabling cells to generate energy efficiently and adapt to stress [4]. Mitochondrial characteristics reflect distinct metabolic phenotypes and modulate immune cell function in a cell type- and activation-specific manner. As lymphocytes, monocytes, and neutrophils transition from quiescent to activated states, their metabolic programs are dynamically dependent on the type, fate, stage, and functional demand, resulting in changes in oxygen demand and mitochondrial respiratory activity. In this context, mitochondrial function in immunometabolism constitutes a central determinant of immune cell activation status and effector function [5–8].

Within the framework of immunometabolism, growing attention has focused on the assessment of distinct mitochondrial respiratory states, because mitochondrial respiration reflects the metabolic competence and ability of immune cells to respond to activation, inflammatory stimuli, and environmental challenges [9]. In particular, basal and maximal mitochondrial respiration provide functional insights into immune cell bioenergetic capacity and oxidative performance, reflecting their ability to sustain and adapt their metabolic responses under physiological and pathological conditions. However, mitochondrial respiratory adaptations are mostly discussed as being modulated through intrinsic signaling pathways, such as cell-intrinsic factors or extracellular nutrient availability, but how extrinsic modulators influence mitochondrial respiration remains unclear [10].

Exercise represents a potent extrinsic modulator by provoking a transient immune challenge through temporary inflammation and metabolic stress [11–13]. Instead, regular physical activity and an active lifestyle are associated with higher cardiorespiratory fitness (CRF), typically expressed as VO_2max. Higher CRF has been shown to enhance immune function, reduce systemic low-grade inflammation, and improve overall health, representing another extrinsic modulator that may support preserved mitochondrial respiratory function and metabolic adaptability of immune cells [14, 15]. However, given the immune system's complexity, the mechanisms linking acute and regular exercise to enhanced immune function

are likely multi-dimensional [16]. In addition to acute exercise and CRF, metabolic risk factors, aging, and disease states constitute additional extrinsic modulators of mitochondrial respiration in immune cells, given their links to mitochondrial dysfunction and low-grade inflammation. In particular, metabolic disturbances associated with metabolic syndrome, including dyslipidemia or elevated blood pressure, have been linked to impaired mitochondrial function and altered cellular energy metabolism [17]. Aging is accompanied by a progressive decline in metabolic flexibility of immune cells, characterized by shifts in immune cell composition, increased low-grade inflammation, and altered mitochondrial regulation, which together may impair bioenergetic resilience [18]. Similarly, in chronic diseases, such as heart failure and obesity, as well as in depression, mitochondrial dysfunction has been closely linked to inflammatory burden and adverse health outcomes [19]. Immune cells' mitochondrial respiration may therefore serve as both a potential therapeutic target to strengthen immune resilience, reduce inflammation, and support long-term health, as well as a potential mechanistic mediator connecting exercise, CRF, metabolic status, and inflammatory processes in health, disease, and aging.

Despite the growing recognition of immunometabolism as a determinant of immune function, experimental evidence in humans directly assessing mitochondrial respiration of immune cells remains scarce. Most available insights derive from animal models, patient cohorts, indirect plasma biomarkers, or skeletal muscle adaptations, and only a few human studies have examined mitochondrial respiration of peripheral blood mononuclear cells (PBMCs) in relation to acute exercise, CRF, metabolic risk, inflammation, aging, and disease. Consequently, it remains unclear whether mitochondrial respiration acts as a mechanistic mediator of immune adaptation across different physiological and pathological contexts. Addressing how extrinsic modulators influence immune cell mitochondrial respiration is therefore critical for understanding changes in immune function, the delay of immunosenescence, and the prevention of chronic disease.

Therefore, this dissertation comprises three complementary parts to address this research gap. The first study investigated how acute exercise and CRF affect $CD4^+$ T cell metabolism in healthy young men. The second study examined mitochondrial respiration of PBMCs in relation to CRF, immunological, and metabolic risk in healthy, older adults. In order to integrate these findings, a narrative review summarized the current evidence on mitochondrial respiration in immune cells in response to acute exercise, regular training, and CRF in health and disease. Together, these studies aim to determine how mitochondrial respiration in immune cells is altered by different extrinsic modulators and how these changes relate to physiological, pathological, and inflammatory intrinsic adaptations.

2 Aim of This Dissertation Thesis

This dissertation aims to assess mitochondrial respiratory states as markers of mitochondrial respiratory function in immune cells. It further investigates how mitochondrial respiratory function is modulated by extrinsic factors, including acute exercise, CRF, and metabolic health. To address this aim, the following research questions are examined in the corresponding scientific publications:

1. How do two different types of acute exercise affect the mitochondrial respiration and metabolic capacity of $CD4^+$ T cells in healthy, young adults? What is the role of CRF, and how does it impact the cellular respiratory and metabolic activity of $CD4^+$ T cells? (Paper I)

2. What is the relationship between mitochondrial respiration in PBMCs, immunological, metabolic markers, and lifestyle factors in the healthy elderly? (Paper II)

3. What is the current evidence on acute and chronic exercise on mitochondrial respiration of PBMCs and their subpopulations? What is the role of CRF on mitochondrial respiration of PBMCs and their subpopulations in healthy vs. diseased participants? (Paper III)

3 Theoretical Background

3.1 Functional Characteristics and Immunometabolism of PBMCs with a Focus on $CD4^+$ T Cells

Innate and adaptive immune cells originate from hematopoietic stem cells in the bone marrow to protect the body from harmful pathogens and can dynamically adapt their metabolic programs in response to signals they receive from other cells or the environment, a process referred to as immunometabolism [20]. This metabolic reprogramming enables immune cells to acquire and maintain their phenotypic and functional state [6, 21]. Metabolism affects the differentiation and function of immune cells, as their activation and effector responses depend on sufficient energy supply and metabolic reprogramming [22, 23]. Immune signaling and cellular metabolism are tightly interconnected, with nutrient availability and metabolite sensing bidirectionally regulating immune cell activation and differentiation [24]. Six main metabolic pathways regulate immunometabolism: glycolysis, tricarboxylic acid (TCA) cycle, amino acid metabolism, pentose phosphate pathway, fatty acid oxidation (FAO), and fatty acid synthesis [2]. Immune cell activation is governed by an integrated signaling framework comprising antigen recognition (signal 1), co-stimulatory molecules on antigen-presenting cells and receptors (signal 2), cytokine signaling (signal 3), and nutrient signals (signal 4) [25]. These signals collectively drive metabolic transitions from a quiescent to a highly active state, characterized by a shift in their predominant energy-producing pathways [23].

Human PBMCs are a mixture of specialized innate and adaptive immune cells, including monocytes, dendritic cells, and lymphocyte subsets such as T cells, B cells, and NK cells. Despite their heterogeneity, PBMCs provide an accessible cross-sectional representation of systemic immune status and respond sensitively to physiological perturbations, making them a widely used model to study immunometabolic adaptation in health and disease [26–28]. Within PBMCs, T cells play a central role in initiating and orchestrating adaptive immune responses and directly eliminating infected or malignant cells [29]. After originating from bone marrow, T cell precursors migrate to the thymus, where they undergo maturation and selection before entering the peripheral circulation [30]. Naïve T cells are antigen-inexperienced, metabolically quiescent cells that primarily rely on oxidative phosphorylation (OXPHOS) fueled by glucose and FAO to efficiently generate ATP [31]. This results in limited nutrient uptake and a predominance of mitochondrial ATP production through complete substrate oxidation. T cell heterogeneity arises from the differentiation stage, memory formation, and functional

polarization [31–33]. Upon antigen recognition, they become activated, proliferate, and differentiate into effector subsets that mediate cytotoxicity, cytokine secretion, and immune regulation, which requires a metabolic reprogramming toward anabolic pathways [25]. This transition is initiated by PI3K-Akt signaling and mediated by activation of the mTOR pathway, which integrates nutrient and growth factor signals to promote glycolysis, glutaminolysis, and lipid synthesis in support of proliferation and cytokine production. Transcriptional regulators, including c-Myc and HIF-1α, further amplify this metabolic shift by enhancing glycolytic gene expression and glucose uptake, ensuring sufficient energy for sustained T cell activation [3]. Effector T cells (Teff) markedly increase glucose uptake through GLUT1 and rely on glycolysis, similar to the Warburg effect in tumor cells, to rapidly generate energy and metabolic intermediates, a less efficient but faster way to generate ATP than OXPHOS. It has been shown that glycolytic rate and mitochondrial respiration increase in T cell activation [32]. After activation, a subset of Teff cells persists as memory T cells (Tmem), enabling rapid responses upon re-exposure to the same antigen [34]. These Tmem can be divided into several subsets, including stem cell-like memory (SCM), central memory (CM), effector memory (EM), and terminal effector memory (EMRA) cells [35]. While SCM and CM subsets are capable of self-renewal and sustain long-term immunological memory, EM and EMRA cells display enhanced effector capacity but reduced proliferative potential [36, 37]. Tmem adopt a more quiescent phenotype characterized by OXPHOS and FAO, which supports longevity and recall capacity. Within this compartment, SCM and CM subsets primarily depend on OXPHOS and FAO to sustain longevity and self-renewal, while EM cells also depend on aerobic glycolysis to enable immediate effector functions [38].

Beyond differentiation and memory status, T cells can be further categorized according to functional specialization. Within $CD4^+$ T cells, the main subpopulations include Th1, Th2, Th9, Th17, Th22, Tfh, and regulatory T cells (Tregs), whereas analogous cytotoxic subsets exist within $CD8^+$ T cells [39]. At the subset level, Th1, Th2, and Th17 cells are associated with pro-inflammatory conditions, predominantly relying on glycolysis for energy and effector function, with upregulation of GLUT1 expression supporting increased glucose uptake and glycolysis [40]. Tregs maintain immune tolerance by suppressing excessive immune responses through inhibitory cytokines (e.g., IL-10, TGF-ß), indicating an anti-inflammatory response [34, 41]. This lineage is defined by FOXP3 and regulated through NF-κB-dependent transcription and epigenetic mechanisms [42, 43]. Both memory (mTregs) and resting (rTregs) subsets preferentially rely on FAO and OXPHOS, maintaining their suppressive function via FOXP3-mediated upregulation of mitochondrial genes and inhibition of glycolytic flux [44, 45].

3.2 Mitochondrial Function and Respiration in PBMCs and CD4$^+$ T Cells

Given that mitochondrial respiration represents a central mechanism of cellular energy metabolism and immune cell function, mitochondria act as central hubs in immunometabolism. Mitochondria are membrane-bound organelles that integrate cellular metabolism, stress responses, and homeostasis maintenance. Beyond ATP generation through OXPHOS, they regulate redox signaling, calcium homeostasis, apoptosis, and immune responses through specific signaling mechanisms. Mitochondria compartmentalize major metabolic pathways, including OXPHOS, the TCA cycle, and FAO. Their structural and functional plasticity enables a rapid adaptation to metabolic demands [7]. Changes in mitochondrial mass and morphology across immune phenotypes are closely linked to their activation state and metabolic specification [32, 46]. Through the regulation of metabolic fluxes, mitochondria shape immune cell fate and effector functions, which is why their bioenergetic role in regulating immune responses is widely appreciated [6, 46]. Mitochondria also adapt to environmental changes, such as hypoxia or nutrient availability, to adjust immune responses, i.e., tumor-derived lactate can reprogram macrophage activation [47]. Especially, immune differentiation and function depend on specific mitochondria-driven programs, including ATP generation from oxidation of nutrients and precursors for synthesis of macromolecules and post-translational modifications (PTMs) [48].

3.2.1 Mitochondrial Metabolism and the Electron Transport System

Mitochondrial energy metabolism is fundamentally driven by the electron transport system (ETS), which is embedded in the inner mitochondrial membrane (IMM) and enables the conversion of reducing equivalents into usable cellular energy. Reducing equivalents (NADH and $FADH_2$) generated during glycolysis, TCA cycle, and FAO are subsequently oxidized within the respiratory chain and donate electrons to the ETS, thereby sustaining oxidative metabolism and regenerating NAD^+ and FAD required for upstream metabolic fluxes [31]. The ETS consists of five multi-subunit protein complexes (complexes I–V) that operate in a coordinated manner to transfer electrons to molecular oxygen. Electrons derived from NADH enter the chain at complex I (NADH dehydrogenase), whereas electrons from $FADH_2$ are transferred via complex II (succinate dehydrogenase) and are transferred through mobile electron carriers, ubiquinone and cytochrome c, to complex IV (cytochrome c oxidase). Ubiquinone serves as a mobile electron carrier within the IMM, shuttling electrons to complex III (ubiquinol cytochrome c oxidoreductase), from where they are passed via cytochrome c to complex IV. At this terminal complex, electrons are transferred to molecular oxygen, resulting

in the formation of water. Electron transport through complexes I, III, and IV is coupled to proton translocation from the mitochondrial matrix into the intermembrane space, generating an electrochemical proton gradient across the IMM. This gradient represents stored potential energy and is utilized by ATP synthase (complex V) to drive the phosphorylation of ADP to ATP as protons flow back into the matrix. This tightly coupled process, referred to as OXPHOS, enables the highly efficient conversion of energy derived from nutrient oxidation into ATP. Full oxidation of glucose or fatty acids through this system yields substantially more ATP than anaerobic metabolic pathways, such as glycolysis, but requires the continuous availability of oxygen as the terminal electron acceptor [31, 49]. In immune cells, respiratory activity adapts to changing energetic and biosynthetic demands during quiescence, activation, and differentiation. In T cells, several in vitro studies have shown that ETS activity supports activation and proliferation in $CD4^+$ T cells. In contrast, inhibition of ATP synthase with oligomycin effectively blocks $CD4^+$ T cell proliferation, underscoring the bioenergetic dependency of immune activation [32, 50]. Specific ETS complexes (e.g., complex I and III) act as sources of mitochondrial reactive oxygen species (ROS), which function as signaling molecules that modulate immune cell activation, cytokine production, and differentiation [46].

3.2.2 Assessment of Mitochondrial Respiratory States

Mitochondrial respiration reflects a central regulator of cellular bioenergetic capacity and metabolic flexibility, in which oxygen consumption is driven by electron flux through the ETS [49]. Measurements of mitochondrial respiration (often depicted as ‘oxygen consumption rate’) allow the assessment of overall metabolic activity, mitochondrial efficiency, and mitochondrial capacity of immune cells under physiological and pathological conditions [10]. High-resolution respirometry (HRR) using Oroboros O2k Oxygraph enables real-time assessment of oxygen consumption in intact or permeabilized cells [51, 52]. Using standardized substrate-uncoupler-inhibitor titration (SUIT) protocols, distinct respiratory states can be quantified by sequential manipulation of the ETS [52]. Within this dissertation, respiratory states represent the primary outcomes derived from Oroboros-based HRR. Basal respiration reflects oxygen consumption under endogenous substrate supply and thus, the current metabolic state of the cell under unstimulated conditions. Inhibition of Complex V with oligomycin allows the assessment of proton leak-associated respiration. The application of chemical uncouplers such as carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) abolishes mitochondrial coupling and reveals maximal respiration, reflecting the maximal electron transfer capacity (ETC) of the ETS independent of ATP demand. Finally, inhibition of complex III with antimycin A enables correction for non-mitochondrial oxygen consumption [10, 52, 53]. Importantly, maximal

respiration must be distinguished from OXPHOS-linked respiration. Whereas OXPHOS reflects ATP-coupled flux constrained by cellular energy demand, maximal respiration is assessed under uncoupled conditions, enabling unrestricted electron flow through the ETS and thereby reflecting the intrinsic maximal ETC, independent of cellular energy demand or ATP turnover. Although non-physiological, maximal respiration yields insights into the intrinsic bioenergetic capacity of immune cells by denoting the respiratory reserve that immune cells can mobilize during acute metabolic challenges, such as immune activation, inflammatory responses, or physical exercise. Taken together, HRR provides mechanistic insights into mitochondrial coupling efficiency and respiratory capacity [10].

3.2.3 Mechanisms of Mitochondrial Respiratory Function in Health & Disease

Beyond electron transfer and ATP production, mitochondrial function and health depend on tightly coordinated quality-control mechanisms that preserve respiratory capacity and maintain metabolic homeostasis [54]. Mitochondrial dynamics, including fission, fusion, biogenesis, and mitophagy, continuously adjust mitochondrial architecture, content, and proteostasis to sustain optimal ETS performance under changing energy demands. These processes ensure the maintenance of key regulatory proteins and IMM integrity, which are essential for preserving coupling efficiency and ETC [54]. The activity of the ETS is further fine-tuned by multiple regulatory mechanisms, including enzyme activity influenced by intracellular metabolite ratios (e.g., NAD^+/NADH, ADP/ATP), substrate availability, and the abundance of respiratory complexes, which collectively determine respiratory flux. Mitochondrial respiratory adaptations in immune cells are coordinated through transcriptional, translational, and posttranslational mechanisms that adjust mitochondrial content, architecture, and functional capacity to changing energetic demands, while controlled ROS production acts as an additional signaling mechanism linking cellular metabolism with immune cell function [48, 49].

Mitochondrial dysfunction develops when the integrity of oxidative metabolism and ETS activity is compromised, resulting in reduced NADH and $FADH_2$ oxidation, impaired redox balance, and diminished ATP synthesis. This process is further aggravated by excessive ROS formation, impaired OXPHOS, and disrupted calcium handling [55, 56]. Early features of mitochondrial dysfunction include altered oxygen consumption and reduced ATP-linked respiration, often accompanied by increased proton leak and diminished ETC. Damaged or insufficiently cleared mitochondria may further propagate inflammation through ROS-dependent activation of NLRP3 inflammasome and amplification of NF-κB-dependent signaling, or through DNA release. Such feed-forward mechanisms link impaired mitochondrial respiration to chronic inflammation, tissue damage, and organ dysfunction [55, 57, 58].

3.3 Insights into Potential Modulators of Mitochondrial Respiratory Function

Mitochondrial respiration in immune cells is shaped by intrinsic metabolic programs and extrinsic environmental cues. Intrinsic modulators include nutrient availability, redox status, oxygen levels, the cellular microenvironment, and endoplasmic reticulum stress signaling. Extrinsic stimuli are behavioral factors as well as pathological conditions leading to adaptations of the mitochondrial respiratory function.

3.3.1 Exercise and Cardiorespiratory Fitness

The immunological response to acute and chronic exercise encompasses dynamic changes in immune cell distribution, cytokine release, and inflammatory signaling [59]. Acute exercise bouts rapidly increase oxygen demand, substrate turnover, catecholamine release, and hemodynamic shear stress, promoting the mobilization of lymphocytes, monocytes, and granulocytes into circulation, resulting in exercise-induced leukocytosis [13, 60]. Subsequent lymphopenia during recovery reflects the redistribution of lymphocytes to peripheral tissues, such as the skin and mucosa, enhancing immune surveillance [61, 62]. This reaction is strongly driven by T EM and NK cells, whereas other lymphocyte subtypes (e.g., B cells) are less responsive [63]. $CD4^+$ and $CD8^+$ T cell numbers transiently increase after acute exercise, with a preferential mobilization of $CD8^+$ T cells, resulting in a reduced CD4:CD8 ratio [15]. While naïve and CM T cells slightly increase, EM and EMRA T cells are mobilized to a greater extent after exercise [64, 65]. These cellular shifts are accompanied by a characteristic cytokine response. Acute exercise induces transient elevations in pro-inflammatory cytokines such as IL-6, TNF-α, IL-8, and IL-1β, promoting immune activation and leukocyte mobilization. IL-6, in particular, shows an early intensity-dependent increase that can reach levels observed during infection or sepsis. Subsequently, a secondary rise in anti-inflammatory mediators such as IL-10 and IL-1ra counterbalances this response, facilitating resolution and tissue recovery [13, 66]. Together, these responses indicate a transient mild pro-inflammatory milieu that can be amplified during eccentric exercise, such as downhill running [66].

From a metabolic perspective, acute exercise alters systemic substrate availability and activates energy sensors such as AMPK, mTOR, HIF-1α, and PPARs, which regulate immunometabolism and are accompanied by transcriptomic changes in genes related to cell death, cytotoxicity, MAP signaling pathways (e.g., MAPK1), T cell surface receptors, and membrane proteins [8, 64]. Despite these responses, studies directly assessing mitochondrial respiration in immune cells report heterogeneous findings. Moderate endurance exercise (30

minutes at 65 - 75% VO_2max) has not consistently been associated with acute changes in mitochondrial respiration in PBMCs and $CD4^+$ T cells immediately post-exercise [16, 67]. Nevertheless, post-exercise immune activation may still occur, as non-stimulated $CD4^+$ T cells isolated after exercise show increased CD25 expression after 24 hours of cell culture [68, 69]. Overall, evidence linking acute exercise to measurable alterations in mitochondrial respiration of PBMCs and subtypes remains limited.

Repetitive exposure to exercise-induced metabolic perturbations promotes long-term adaptations. Regular exercise alters the metabolic environment of peripheral immune cells, such as T cells, by shifting them toward anti-inflammatory phenotypes and enhancing their metabolic flexibility [8, 70]. Repeated exercise bouts further stimulate T cell mobilization, turnover, and renewal, favoring a higher proportion of naïve and CM T cells while reducing EMRA T cells [71]. In addition, chronic training may enhance mitochondrial function and metabolic efficiency, supporting increased FAO and glucose utilization during rest and subsequent exercise sessions [70].

Physical activity (PA) describes any bodily movement that increases energy expenditure, whereas CRF, commonly assessed via VO_2max, reflects an integrative marker of cardiovascular, pulmonary, and muscular performance that is strongly influenced by habitual PA and independently predicts morbidity, mortality, and quality of life [72–74]. As CRF plays an important role in long-term adaptations to exercise, higher CRF has been linked to a more favorable immune profile, characterized by lower circulating IL-6 and TNF-α and higher levels of IL-10 and IL-33. Higher CRF and regular endurance training are also linked to higher frequencies and enhanced regulatory capacity of Tregs, particularly mTregs, accompanied by lower proportions of mTeff and pro-inflammatory monocytes [75, 76]. In monocytes, these anti-inflammatory benefits may partly depend on individual CRF, as higher VO_2max levels exhibit enhanced expression of genes such as TLR-4, IKK, PGC-1α, and PPAR-γ, which may contribute to improved regulation of inflammatory responses through suppression of NF-κB signaling [77]. These observations support the immunoregulatory role of CRF in maintaining a low inflammatory burden by enhancing immune adaptability and inflammation control, thereby mitigating immunometabolic consequences of inactivity, obesity, and aging [8, 70]. Such adaptations are associated with reduced chronic disease risk, improved physical performance, and healthier aging [60–62]. However, whether acute exercise, long-term exercise, and higher CRF translate into sustained adaptations in mitochondrial respiration of immune cells remains unclear, highlighting the need to better understand its role as a potential modulator and mechanistic link between PA, immune function, and health.

3.3.2 Immunometabolism in the Elderly

Aging is accompanied by a progressive decline in metabolic flexibility of immune cells, driven in part by decreased hematopoietic stem cell function followed by impaired self-renewal and differentiation. These alterations contribute to structural and functional remodeling of the immune system, termed immunosenescence, which is characterized by reduced immune adaptability followed by increased susceptibility to infections and age-related diseases [78]. Alterations affecting both innate and adaptive immunity promote persistent low-grade inflammation and functional decline [79]. While the total T cell pool remains largely stable, the composition of T cell subsets changes markedly. A higher proportion of $CD8^+$ T cells and a reduced $CD4^+$ T cells lower the CD4:CD8 ratio, a central feature of the immune risk profile [80]. In parallel, naïve $CD4^+$ and $CD8^+$ T cells decrease due to thymic involution and chronic antigenic stimulation, whereas highly differentiated and senescent T cells accumulate [81]. These compositional changes are accompanied by a progressive functional decline, including weakened TCR signaling, diminished proliferation, and altered cytokine patterns contributing to impaired immune surveillance and a chronic low-grade inflammation (“inflammaging”). The disturbed communication between $CD4^+$ T cell subsets and their cytokine milieu plays a central role in this process [79, 82]. Mitochondrial dysfunction represents a central hallmark of aging, cellular senescence, and numerous age-related diseases [83, 84]. Age-associated alterations, which can cause mitochondrial dysfunction, include reduced mitochondrial biogenesis, enhanced fission with reduced fusion dynamics, accumulation of mitochondrial DNA (mtDNA) mutations, reduced NAD^+/NADH ratio, calcium overload, dysregulated nutrient-sensing pathways (e.g., mTOR, AMPK), and excessive ROS production [83, 85]. These structural and functional deteriorations impair ATP synthesis and promote redox imbalance, thereby activating inflammatory signaling pathways such as NF-κB and contributing to immunosenescence and low-grade inflammation [70]. Given the energetic demands of immune cells, mitochondrial alterations directly affect immunometabolism and function (see chapter 3.2). At the metabolic level, aging immune cells exhibit reduced mitochondrial efficiency, lower OXPHOS capacity, and altered energy utilization, leading to a metabolic shift with disturbed mitochondrial function, followed by impaired energy metabolism, decreased respiratory capacity, and decreased membrane potential. This decline in oxidative capacity is accompanied by altered metabolic flexibility and a greater reliance on glycolytic pathways. [83, 86]. Collectively, aging positions mitochondrial dysfunction as a mechanistic link between immunosenescence and age-related disease development. Although a healthy and physically active lifestyle is suggested to reduce the risk of chronic and inflammatory diseases during

aging, regular PA and higher CRF may mitigate age-related immunometabolic alterations by supporting mitochondrial function, preserving immune adaptability, and thus, reducing low-grade inflammation [87]. In older adults, higher CRF is associated with a lower accumulation of senescent T cells and a greater proportion of naïve T cells, indicating a more favorable inflammatory risk profile, and underscoring the importance of a physically active lifestyle on the aging immune system [71, 79]. However, whether these adaptations directly modulate preserved or enhanced mitochondrial respiration of immune cells remains unclear.

3.3.3 Inflammation and Chronic Disease

Inflammation represents another potential modulator of mitochondrial respiratory adaptations. In physiological conditions, inflammation is a tightly regulated host defense response to infection or tissue injury, characterized by transient cytokine production and coordinated recruitment of innate immune cells to restore tissue homeostasis; however, persistent stimuli or impaired resolution mechanisms drive chronic inflammation associated with the development of metabolic and degenerative diseases. Activation of NF-κB and inflammasome-dependent signaling cascades promotes the production of pro-inflammatory cytokines and ROS, directly affecting mitochondrial bioenergetics. One of the earliest manifestations of inflammation-induced mitochondrial dysfunction is a slight and reversible inhibition of mitochondrial respiratory function, particularly impaired ETS activity, resulting in reduced OXPHOS efficiency and diminished ATP synthesis [57, 88]. Concurrently, excessive ROS production can activate NLRP3 inflammasome, thereby reinforcing inflammatory signaling in a self-amplifying feedback loop [55, 89]. In chronic diseases, such as metabolic syndrome, diabetes, neurodegenerative, cardiovascular, and malignant diseases, persistent cytokine exposure disrupts mitochondrial dynamics by altering fusion-fission balance, reducing mitochondrial biogenesis, and promoting mitochondrial fragmentation and mtDNA damage [57]. Inflammatory mediators further induce a metabolic reprogramming from OXPHOS toward aerobic glycolysis, particularly in activated immune cells, leading to lactate accumulation and reduced bioenergetic efficiency [90]. Collectively, these mechanisms establish a vicious cycle in which inflammation impairs mitochondrial respiration and, thus, mitochondrial dysfunction in turn perpetuates systemic inflammation and organ dysfunction. These considerations underscore that inflammation and chronic disease constitute critical pathological modulators of mitochondrial respiration in immune cells, whereas other modulators, such as CRF, may counteract inflammatory bioenergetic impairment and restore mitochondrial homeostasis.

4 Synopsis

This synopsis integrated the findings of three complementary studies that collectively address mitochondrial respiratory states of immune cells as central markers of mitochondrial respiratory function and immunometabolic adaptation. By combining experimental, cross-sectional, and narrative approaches, this dissertation provides a multi-level perspective on how exercise, CRF, aging, and metabolic risk shape immune cell bioenergetics.

4.1 Conceptual Framework of this Dissertation Thesis

This cumulative dissertation is conceptually centered on mitochondrial respiratory states, mainly basal and maximal respiration, to capture immunometabolic adaptations across different physiological contexts. Basal respiration reflects the prevailing metabolic state of immune cells under steady-state conditions, whereas maximal respiration provides insight into the upper limit of mitochondrial performance. Together, these parameters allow the assessment of metabolic flexibility and adaptive potential across different physiological challenges, including acute exercise, CRF, aging, disease, and inflammation. Within this framework, immune cell mitochondrial respiration is considered a functional interface linking systemic challenges to cellular immune function. The included studies address complementary dimensions of this framework by investigating acute versus chronic effects of exercise, different immune cell populations, and varying levels of physiological complexity.

4.2 Overview of the Included Studies

This dissertation investigates the respiratory and metabolic adaptations of $CD4^+$ T cells and PBMCs in response to different physiological challenges. The underlying purpose is to explore the effects of exercise, CRF, and metabolic and inflammatory factors on immune cell mitochondrial respiration. The three included studies differ in design and scope but collectively contribute to a coherent investigation of immunometabolic regulation at the cellular level.

4.2.1 Study Section I (Gebhardt et al., 2024)

Study I examined respiratory and metabolic changes of $CD4^+$ T cells following acute endurance exercise and their association with CRF. Using a controlled cell culture experiment, freshly isolated $CD4^+$ T cells from the rested participant were incubated in autologous serum obtained before and three hours after 45 minutes at 70% of VO_2max level (LR) and downhill running (DR). Mitochondrial respiration served as the primary outcome, complemented by transcriptomic and metabolomic analyses, while immune cell subpopulations and serum

cytokines were assessed as secondary outcomes. The study aimed to differentiate the effects of concentric LR and eccentric DR endurance exercise stimuli and to evaluate whether CRF modifies cellular immunometabolic responsiveness.

4.2.2 Study Section II (Gebhardt et al., 2025)

Study II investigated mitochondrial respiration of PBMCs in healthy older adults to characterize age- and lifestyle-associated variations under steady-state conditions. This cross-sectional study focused on older individuals without pre-existing diseases to capture physiological, not pathological, aging. The study explored associations between mitochondrial respiration of PBMCs, immune cell subpopulations, cytokines, metabolic markers, and lifestyle-related factors, including CRF. This approach aimed to identify links between immune cell bioenergetics, immunological composition, and metabolic resilience in healthy older adults.

4.2.3 Study Section III (Gebhardt & Krüger, 2024)

Study III comprises a narrative review summarizing current evidence on mitochondrial respiration of immune cells in the context of acute exercise, chronic exercise, and CRF in health and disease. Studies examining PBMCs and their subtypes, lymphocytes, and isolated T cells, B cells, NK cells, platelets, and neutrophils across healthy and diseased populations were included. The review contextualizes the experimental findings of Studies I and II within the broader scientific field of immunometabolism and highlights methodological and conceptual gaps in the existing literature.

4.3 Integrated Interpretation of Findings

4.3.1 Acute Exercise and CRF as Modulators of Mitochondrial Respiratory Adaptation in $CD4^+$ T cells

Acute exercise alone induced only minor immediate changes in mitochondrial respiration of $CD4^+$ T cells, despite clear systemic inflammatory responses in both DR and LR. In contrast, higher CRF was consistently associated with enhanced mitochondrial respiratory capacity, particularly maximal respiration, in $CD4^+$ T cells. These findings are supported by complementary transcriptomic and metabolomic data.

These findings suggest that immune cell bioenergetic adaptations are not driven by single exercise bouts but rather reflect cumulative exposure to repeated metabolic challenges that accumulate over time and then drive chronic adaptations. CRF therefore represents a functional surrogate of long-term immunometabolic adaptation, influencing oxidative metabolism and respiratory adaptations in $CD4^+$ T cells.

4.3.2 Mitochondrial Respiration of PBMCs and Metabolic Markers in Healthy Aged Individuals

In healthy older adults, mitochondrial respiration of PBMCs was associated with immune cell composition, inflammatory markers, and metabolic parameters, highlighting its role as an indicator of immunometabolic resilience. A higher maximal respiration of PBMCs was associated with a greater proportion of $CD4^+$ T cells, $CD8^+$ T cells, naïve $CD4^+$ T cells, and lower CD4EM cells. The analysis for cytokines and intracellular signaling revealed that the basal respiration was negatively associated with IL-17 and TNF-α and was positively associated with myoglobin concentrations. A positive association between maximal respiration and serum levels of IL-8, ICAM-1, VEGF, and STAT3 expression was found.

These associations suggest that preserved mitochondrial respiratory function may buffer age-related immune alterations, even in the absence of overt disease. PBMCs' mitochondrial respiration reflects resilience in healthy older adults, linking higher respiratory capacity to naïve $CD4^+$ T cells and lower pro-inflammatory cytokines. Notably, mitochondrial respiration of PBMCs was more strongly related to immunological and metabolic markers than to CRF, underscoring the multifactorial regulation of immune cell bioenergetics during aging.

4.3.3 Mitochondrial Respiration of PBMCs and Subtypes as an Integrative Marker of Acute Exercise, CRF, and Inflammation in Health and Disease

The narrative review supports the experimental findings by demonstrating that immune cell mitochondrial respiration is sensitive to both physiological and pathological conditions. While acute exercise shows heterogeneous effects, higher CRF and long-term training are frequently associated with enhanced mitochondrial respiration in immune cell subpopulations. When comparing patients with healthy controls, reduced CRF, basal, and maximal mitochondrial respiration of PBMCs were found. In particular, studies revealed negative associations between mitochondrial respiration of PBMCs with inflammation markers (e.g., TNF-α, CRP, IL-6) and disease severity.

Together, these observations position immune cell mitochondrial respiration as an integrative marker linking physical fitness, inflammatory burden, and disease severity. Given its association with enhanced mitochondrial capacity in immune cells, CRF may serve as a marker of metabolic resilience. In contrast, disease states are frequently characterized by impaired immune cell respiration. This contrast highlights the potential role of immune cell mitochondrial dysfunction as a mediator between reduced fitness, chronic inflammation, and disease severity.

5 Publications

The results of this dissertation were published in two original articles and one narrative review, which are listed in the following subchapters 5.1, 5.2, and 5.3. The first paper (study section I) was published in Medicine & Science in Sports & Exercise [91]. The second paper (study section II) was published in the Journal of Cellular Physiology [92]. The third paper (study section III) was published in the German Journal of Sports Medicine [93].

5.1 Respiratory and Metabolic Responses of $CD4^+$ T cells to Acute Exercise and Their Association with Cardiorespiratory Fitness

Gebhardt K, Hebecker A, Honekamp C, Nolte S, Barthkuhn M, Wilhelm J, Klatt S, Weyh C, Sommer N, and Krüger K (2024). Respiratory and Metabolic Responses of CD4+ T Cells to Acute Exercise and Their Association with Cardiorespiratory Fitness. Medicine & Science in Sports & Exercise, 56(10), 1882-1892.

DOI: https://doi.org/10.1249/MSS.0000000000003483

Supplementary Material: http://links.lww.com/MSS/D42

Specification of the authors' contributions:
Conceptualization: Kristina Gebhardt, Natascha Sommer, Karsten Krüger; **Development and design of methodology:** Kristina Gebhardt, Karsten Krüger**; Investigation:** Kristina Gebhardt, Anne Hebecker, Celine Honekamp, Svenja Nolte; **Formal analysis:** Kristina Gebhardt, Marek Bartkuhn, Jochen Wilhelm, Stephan Klatt, Christopher Weyh; **Writing – Original Draft:** Kristina Gebhardt, Karsten Krüger; **Visualization:** Kristina Gebhardt; **Writing – review & editing:** Kristina Gebhardt, Marek Bartkuhn, Jochen Wilhelm, Stephan Klatt, Christopher Weyh, Natascha Sommer, Karsten Krüger; **Funding acquisition:** Natascha Sommer, Marek Bartkuhn, Jochen Wilhelm, Stephan Klatt, Karsten Krüger

5.2 PBMCs Mitochondrial Respiration and Its Relation to Immunity, Fitness, and Metabolic Risk in the Healthy Elderly

Gebhardt K, Hebecker A, Sommer N, Ringseis R, Eder K, Huber M, Raifer H, Krüger K, and Weyh C (2025). PBMCs Mitochondrial Respiration and Its Relation to Immunity, Fitness, and Metabolic Risk in the Healthy Elderly. Journal of Cellular Physiology, 240(9), e70096.

DOI: https://doi.org/10.1002/jcp.70096

Supplementary Material: https://onlinelibrary.wiley.com/doi/10.1002/jcp.70096

Specification of the authors' contributions:

Conceptualization: Kristina Gebhardt, Magdalena Huber, Natascha Sommer, Robert Ringseis, Klaus Eder, Karsten Krüger, Christopher Weyh; **Development and design of methodology:** Kristina Gebhardt**; Investigation:** Kristina Gebhardt, Anne Hebecker, Hartmann Raifer, Robert Ringseis, Christopher Weyh; **Formal analysis:** Kristina Gebhardt, Christopher Weyh; **Validation:** Hartmann Raifer, Robert Ringseis, Christopher Weyh; **Writing – Original Draft:** Kristina Gebhardt, Karsten Krüger, Christopher Weyh; **Visualization:** Kristina Gebhardt; **Writing – review & editing:** Kristina Gebhardt, Robert Ringseis, Klaus Eder, Karsten Krüger, Christopher Weyh; **Funding acquisition:** Magdalena Huber, Natascha Sommer, Robert Ringseis, Klaus Eder, Karsten Krüger

Check for updates

Journal of Cellular Physiology

WILEY

Journal of Cellular Physiology

RESEARCH ARTICLE OPEN ACCESS

PBMCs Mitochondrial Respiration and Its Relation to Immunity, Fitness, and Metabolic Risk in the Healthy Elderly

Kristina Gebhardt[1] | Anne Hebecker[1] | Natascha Sommer[2] | Robert Ringseis[3,4] | Klaus Eder[3,4] | Magdalena Huber[5] | Hartmann Raifer[5] | Karsten Krüger[1] | Christopher Weyh[1]

[1]Department of Exercise Physiology and Sports Therapy, Institute of Sports Science, Justus-Liebig-University, Giessen, Germany | [2]Department of Internal Medicine, Universities of Giessen and Marburg Lung Center (UGMLC), Member of the German Center for Lung Research (DZL), Excellence Cluster Cardio-Pulmonary Institute (CPI), Justus-Liebig University, Giessen, Germany | [3]Institute of Animal Nutrition and Nutrition Physiology, Justus Liebig University Giessen, Giessen, Germany | [4]Center for Sustainable Food Systems, Justus Liebig University Giessen, Senkenbergstraße 3, Giessen, Germany | [5]Institute for Systems Immunology, Center for Tumor and Immunology, Marburg, Germany

Correspondence: Karsten Krüger (karsten.krueger@sport.uni-giessen.de)

Received: 3 February 2025 | **Revised:** 19 August 2025 | **Accepted:** 17 September 2025

Funding: This study was funded by the Forschungscampus Mittelhessen, Flexi Fund Project number 20121_1_1_10.

Keywords: aging | cell respiration | immune system | inflammation | mitochondria | peripheral blood mononuclear cells | physical fitness

ABSTRACT

Mitochondrial function plays a central role in regulating immunological and metabolic processes, particularly during successful aging. This cross-sectional study aimed to investigate associations between mitochondrial respiration of peripheral blood mononuclear cells (PBMCs; MR_{PBMC}) and key markers of immune function, systemic inflammation, and metabolic health in a cohort of healthy older adults. Sixteen healthy, physically active participants aged > 55 years (male: $n = 9$; female: $n = 7$; age: 64 ± 3.7 years; BMI: 24.3 ± 2.9; VO_{2peak}: 31.1 ± 8.8 mL/min/kg) were recruited. Participants were tested for their maximal oxygen uptake (VO_{2peak}) as well as cardiovascular and metabolic risk factors. Venous fasting blood samples were collected. For further analysis, MR_{PBMC} was measured using the Oroboros O2k-Oxygraph. T cell subsets were analyzed by flow cytometry, serum cytokines by LUMINEX assays, and gene expression by qPCR analysis. We found positive associations between basal and maximal $MR_{PBMC,}$ and the percentage of $CD4^+$ T cells, with a notable link to naïve $CD4^+$ T cells ($p < 0.05$). Maximal MR_{PBMC} was negatively associated with proportion of effector memory $CD4^+$ T cells ($p < 0.05$). Basal MR_{PBMC} showed negative associations with pro-inflammatory serum cytokine tumor necrosis factor alpha (TNF-α), while maximal MR_{PBMC} was positively associated with interleukin 8 (IL-8), intercellular adhesion molecule 1 (ICAM-1), and vascular endothelial growth factor (VEGF) ($p < 0.05$). Intracellular signaling markers, including mRNA level of signal transducer and activator of transcription 3 (STAT3), also showed positive associations with maximal MR_{PBMC} ($p < 0.05$). No correlations were found for variables such as cardiorespiratory fitness, IL-6, and IL-10. In conclusion, PBMC mitochondrial bioenergetics are linked to T cell subpopulations and systemic inflammation in healthy older adults. Higher mitochondrial respiration reflecting better mitochondrial function favors a more naïve $CD4^+$ T cell distribution. In contrast, lower mitochondrial function was observed in individuals with a more pro-inflammatory profile, suggesting a potential relationship between immune status and mitochondrial bioenergetics in older adults.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2025 The Author(s). *Journal of Cellular Physiology* published by Wiley Periodicals LLC.

10974652, 2025, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jcp.70096, Wiley Online Library on [01/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

1 | Introduction

The physiology of aging encompasses a complex array of biological processes that contribute to the gradual decline in functional capacity observed in living organisms over time (J. Guo et al. 2022; Y. Li et al. 2024). Aging is marked by significant metabolic changes, including alterations in overall energy production, nutrient sensing, and cellular mechanisms. These metabolic shifts are often associated with increased systemic inflammation and weakened immunity (Chen et al. 2022; Yang et al. 2024). Central to these processes are mitochondria, whose dysfunction is increasingly recognized as a pivotal factor in metabolic and immune aging (Harrington et al. 2023; Memme et al. 2021). Due to their role in energy metabolism, mitochondria are important modulators of immune responses. For example, activation of T cells involves increased glucose and glutamine oxidation, and in particular glucose oxidation, while oxidative phosphorylation (OXPHOS) remains necessary for functional differentiation (Almeida et al. 2016; Conte et al. 2020; O'Neill et al. 2016). After their activation, immune cells switch back to OXPHOS, for which integrated and functionally stable mitochondria are important to restore the immune balance (O'Neill et al. 2016). Accordingly, mitochondrial respiration, particularly basal and maximal respiration, serves as a critical indicator of mitochondrial function and cellular energy production, which are directly linked to an individual's physiological performance and ability to adapt to physical stress. These measures reflect the cell's capacity to generate energy efficiently under varying conditions, making them essential for understanding overall metabolic health (Avram et al. 2022; Divakaruni and Jastroch 2022; Yu and Pekkurnaz 2018).

In senescent cells, mitochondria undergo structural changes that are often associated with increases in their size and volume, primarily due to the accumulation of dysfunctional mitochondria from alterations in the mitochondria-specific autophagy ('mitophagy') degradation process. These age-related changes in mitochondrial turnover as well as a NAD^+/NADH imbalance, mitochondrial Ca^{2+} overload, mtDNA mutations, and altered nutrient signaling can cause mitochondrial dysfunction, which can be defined as a decreased respiratory capacity per mitochondrion and decreased mitochondrial membrane potential (Miwa et al. 2022). These processes are followed by an impaired energy metabolism, which is related to changes in the six main metabolic pathways that regulate metabolism in immune cells: glycolysis, tricarboxylic acid (TCA) cycle, amino acid metabolism, pentose phosphate pathway, fatty acid oxidation (FAO), and fatty acid synthesis (Conte et al. 2020; O'Neill et al. 2016).

Not only age, but also various inflammatory diseases seem to impair the metabolic capacity of mitochondria in immune cells often reflected in an altered immune cell respiration. Recent studies showed these impaired mitochondrial bioenergetics of various immune cells in different chronic and inflammatory diseases compared to healthy controls (Krüger and Gebhardt 2024). PBMCs represent mononuclear immune cells composed of monocytes, dendritic cells, and lymphocytes (including T, B, and NK cells). In PBMCs, Sommer et al. (2022) found a negative correlation between mitochondrial respiration and disease severity in patients with pulmonary arterial hypertension. Similarly, P. Li et al. (2015) detected lower maximal respiration and negative correlations between mitochondrial respiratory function and inflammatory cytokines and cardio-metabolic risk factors.

In contrast to the negative effects of aging, a healthy lifestyle seems to have a positive impact on the development of chronic and inflammatory diseases. In particular, a nutritious diet, regular physical activity, and maintaining good mental health can significantly delay the aging process (X. Li et al. 2023). Tyrrell et al. (2015) revealed associations between PBMC bioenergetics with key physical markers of aging, reduced physical function, muscle strength and quality, and higher plasma IL-6 levels in obese older adults. While there is evidence that long-term training interventions, such as endurance exercise or high-intensity interval training, are suitable for improving mitochondrial respiration of immune cells (Krüger and Gebhardt 2024), other studies have shown improvements in skeletal muscle mitochondria bioenergetics (Menshikova et al. 2006; Tonkonogi et al. 2000). However, in healthy individuals, PBMC mitochondrial function does not necessarily reflect skeletal muscle mitochondrial function (Hedges et al. 2019). Yet, in clinical populations such as patients with mitochondrial disorders, PBMCs may serve as a surrogate marker of skeletal muscle mitochondrial dysfunction (Alonso et al. 2021).

Therefore, the present study aimed to explore the associations between mitochondrial respiration in peripheral blood mononuclear cells (PBMCs) and key markers of immune function, systemic inflammation, and metabolic health in a cohort of healthy older adults. Recognizing the central role of mitochondrial bioenergetics in regulating immunometabolic processes during aging, we specifically sought to examine how basal and maximal mitochondrial respiratory capacity relate to the distribution of T cell subpopulations, circulating cytokine levels, and intracellular signaling pathways. Furthermore, we investigated whether mitochondrial respiration correlates with cardiorespiratory fitness and metabolic risk factors, including glucose metabolism and body composition. By focusing on a particularly healthy, medication-free, and non-diseased cohort, we aimed to identify physiological variation in mitochondrial and immune parameters that may be obscured in clinical populations. Given the exploratory nature of this study, we applied a cross-sectional study design to capture interindividual differences under steady-state conditions. This approach was chosen to generate hypotheses regarding potential biological interactions that may inform future longitudinal or interventional studies targeting mitochondrial resilience and immune function in aging populations.

2 | Materials & Methods

2.1 | Study Design and Participants

In this cross-sectional study, 16 participants (9 males; 7 females, age: 64 ± 3.7 years; BMI: $24.26 \pm 2.9\ kg/m^2$) were included. These participants form a subgroup of the Giessen Immune aging Study cohort, which has been thoroughly described in a

10973452, 2025, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jsp.70096, Wiley Online Library on [01/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

previous publication, including details on baseline characteristics and endurance performance (Böttrich et al. 2024). All participants were in good health and free from any acute illnesses, including infections or injuries. Recruitment for this study occurred between August 2020 and December 2021. Inclusion criteria required participants to be over 55 years old, with women being postmenopausal. Exclusion criteria included excessive alcohol consumption (> 2 drinks/day), smoking, BMI < 18.5 kg/m², history of cardiovascular (e.g., myocardial infarction), neurological (central/peripheral), obstructive pulmonary, or metabolic diseases (diabetes type 1/2), as well as systemic illnesses (e.g., cancer, arthritis, hepatitis, HIV, autoimmune diseases). Participants were also excluded if they had used immunomodulatory medications within the past 12 weeks or received treatment for specific diseases. The medical ethics committee of the Justus-Liebig-University Giessen approved this study (AZ 100/20). All experimental procedures were performed according to the Declaration of Helsinki, and all participants gave written informed consent before enrolment. Participants were instructed to refrain from exercising the day before the appointment. In case of acute illness (e.g. respiratory infection), the appointment was postponed until full recovery. Fasting venous blood samples were collected between 08:00 and 10:00 am. A volume of 7.5 mL whole blood was drawn into serum gel CAT monovettes and 7.5 mL into K3E-EDTA tubes. Serum was separated and stored at −80°C for cytokine analysis, while anticoagulated whole blood was processed for mononuclear cell isolation. For flow cytometry, 7.5 mL lithium-heparin (LH) tubes were used. Body composition (FFM%) was assessed via bioelectrical impedance analysis (BIACORPUS RX 4004 M, MEDI CAL HealthCare GmbH). Cardiopulmonary exercise testing was performed on an electrically braked cycle ergometer (Excalibur Sport®, Lode) using individual ramp protocols to reach maximum load within 15 min. Maximum voluntary grip strength of the dominant hand was assessed using a hand dynamometer (Baseline® Hydraulic Hand Dynamometer LiTE®, Fabrication Enterprises Inc., US). Anthropometric data are summarized in Table 1.

2.2 | Isolation of Peripheral Blood Mononuclear Cells

To isolate PBMCs, fresh peripheral blood was mixed with an equal volume of phosphate-buffered saline (PBS) and placed on top of EasySep in SepMate 50 mL tubes. After centrifugation at 1200 × g for 10 min, the upper layer was carefully discarded. The isolated cells were then washed and centrifuged again at 300 × g for 8 min. Subsequently, the PBMCs were resuspended in Bambanker® freezing medium containing 10% dimethyl sulfoxide (DMSO) and frozen. The cells were stored at −80°C for later analysis. Samples were collected between December 2021 and March 2022 and stored for approximately 6 months until measurement.

2.3 | High Resolution Respirometry of PBMCs

Frozen PBMCs were thawed at 37°C, washed twice in RMPI 1640 (Gibco, UK) containing 10% FBS (Gibco, UK), then pelleted, and resuspended in mitochondrial respiration media (89 mL RPMI, 1 mL Penicillin-Streptomycin + 10 mL Hepes (0.2% of Hepes Stock Solution). After thawing, PBMC viability was assessed using the CASY Cell Counter and Analyzer System (OLS OMNI Life Science, Germany). This system determines cell viability based on electrical current exclusion, providing a reliable and label-free measure of viable versus nonviable cells. Only samples with a viability ≥ 85% were used for subsequent high-resolution respirometry. Afterward, mitochondrial respiration of PBMCs was determined using an Oroboros-2-k oxygraph (Oroboros Instruments, Innsbruck, Austria) for high-resolution-respirometry (HRR) by using a substrate-uncoupler-inhibitor titration (SUIT) protocol. For each participant, experiments were performed once as singlets at 37°C in a 0.5 mL chamber filled with 4 to 6 $\times 10^6$ PBMCs. Air and background calibrations were performed as recommended. First, the initial respiratory rate (basal respiration) was measured. For quality control, we applied oligomycin (2 μL of 1,25 mM oligomycin) to inhibit ATP synthase and measure LEAK respiration. To assess mitochondrial coupling efficiency, the ratios Leak/ETS (L/E) and (ETS−Leak)/ETS (E − L/E) were calculated. The mean value for L/E was 0.310 (SD = 0.14; 95% CI: 0.23–0.38), and for E − L/E 0.69 (SD = 0.14; 95% CI: 0.61–0.76), indicating preserved mitochondrial coupling and intact membrane integrity in PBMCs. Afterward, stepwise titration of 2 μL of 1 mM carbonyl cyanide p-trifluoro-methoxyphenyl hydrazone (FCCP) was conducted to measure the maximal (uncoupler-stimulated) mitochondrial respiratory capacity. Last, 4 μL of 5 mM antimycin A was titrated to shut down complex III of the ETC. Data were recorded in DatLab version 4.3 (Oroboros Instruments, Innsbruck, Austria). A typical primary recorded experiment is shown in the Supporting Material Figure S1.

TABLE 1 | Anthropometric data shown in mean ± SD with maximum, minimum, and range; $n = 16$ participants (male = 9) were used.

	Mean + SD	Maximum	Minimum	Range
Age	64 ± 3.72	73	59	14
BMI	24.26 ± 2.89	30.57	19.26	11.32
FFM (%)	73.31 ± 6.64	86.09	65.33	20.76
VO_{2peak}	31.06 ± 8.83	49	23	26
Male:	36.00 ± 8.87	27	49	22
Female:	24.71 ± 2.50	30	23	7

Abbreviations: BMI, body-mass-index; FFM, fat-free body mass; VO_{2peak}, maximal oxygen uptake.

10973652, 2025, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jcp.70096, Wiley Online Library on [01/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

2.4 | T Cell Phenotyping by Flow Cytometry

To analyze T cells and their subsets, frozen PBMCs were thawed at 37°C, washed twice in RPMI 1640 (Gibco, UK) containing 10% FBS (Gibco, UK), and then pelleted. The cells were resuspended in PBS at a concentration of 1×10^6 cells/mL. The cells were incubated in the dark at room temperature for 20 min with an optimized amount of each antibody (CD3 (FITC), CD4 (AF700), CD25 (APC), CD45RO (PerCP/Cyanine5.5), CD197/CCR7 (BV421), and CD127 (PE)). For the gating strategy to identify regulatory T cells (T_{regs}) and their subsets, lymphocytes were first selected based on forward scatter/side scatter (FSC/SSC) in a dot plot, with a minimum of 250,000 events per tube. Singlets were identified within the lymphocyte gate. A live/dead stain was performed using Zombie Aqua™ to identify viable lymphocytes (BioLegend Inc., San Diego, CA). Among these live cells, the frequency of $CD3^+CD4^+$ cells was evaluated. T effector cells (T_{eff}) were gated as $CD4^+CD25^+CD127_{high}$, while T_{regs} were defined as $CD4^+CD25^+CD127_{low}$. T_{reg} subsets were further gated as resting-T_{regs} (rT_{regs}) ($CD45RO^-CCR7^+$) and memory-T_{regs} (mT_{regs}) ($CD45RO^+CCR7^-$). The ratio of T_{regs} to T_{eff} was calculated by dividing the total number of T_{regs} by the total number of T_{eff} cells. Flow cytometry analysis was performed using a CytoFLEX S (Beckman Coulter) and Kaluza analysis software 2.1 (Beckman Coulter), with compensation applied for spectral overlap when using multiple colors. For the immune aging panel, PBMCs were thawed, washed, and rested in pre-warmed RPMI/5% AB serum for 2 h. The staining for the second panel (Immune-aging panel) was performed immediately after the resting period using the Zombie NIR fixable viability kit, CD4 (AF700), CD8 (BV510), CD27 (PE), CD28 (BV421), CCR7 (PE/Cy7), CD45RA (PerCP), CD95 (FITC), and PD1 (APC), all from BioLegend (BioLegend Inc., San Diego, CA). For the immune aging panel, $CD4^+$ and $CD8^+$ T cells were gated after identifying live cells using the Zombie NIR fixable viability kit. T cells were then classified into subsets based on their CCR7 and CD45RA expression: naïve T cells ($CD45RA^+CCR7^+$), effector memory (EM) T cells ($CD45RA^-CCR7^-$), central memory (CM) T cells ($CD45RA^-CCR7^+$), and effector memory cells re-expressing CD45RA (TEMRA) T cells ($CD45RA^+CCR7^-$).

2.5 | Determination of Serum Cytokines

Serum samples were stored at −80°C until they were ready for analysis. Protein levels were assessed using a Magnetic Luminex Assay (Bio-Techne, Abingdon, Oxon, UK) and the Magpix Luminex instrument (Luminex Corp, Austin, TX, USA), following the manufacturer's guidelines. The LUMINEX assays included the following cytokines: Adiponectin, matrix metallopeptidase 2 (MMP2), matrix metallopeptidase 9 (MMP9), myeloperoxidase (MPO), myoglobin, carbon tetrachloride (CCL4), chemokine ligand 13 (CXCL13), chemokine ligand 9 (CXCL9), growth/differentiation factor 15 (GDF15), tenascin-C, galectin, intercellular adhesion molecule 1 (ICAM1), interferon-gamma (IFN-γ), interleukin 10 (IL-10), interleukin 15 (IL-15), interleukin 17 (IL-17), interleukin 18 (IL-18), interleukin 1 receptor antagonist (IL-1ra), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), tumour necrosis factor alpha (TNF-α), and vascular endothelial growth factor (VEGF). The list of their assay ranges is presented in Supporting Material Table S1.

2.6 | RNA Extraction and qPCR analysis

For the isolation of PBMCs, total RNA was extracted using TRIzol reagent (Invitrogen, Karlsruhe, Germany), following the manufacturer's instructions. RNA concentration and quality were assessed using an Infinite 200 M microplate reader (Tecan, Mainz, Germany) equipped with a NanoQuant plate. The cDNA synthesis was performed as previously described in Keller et al. (2012) and qPCR analysis was conducted on a Rotor-Gene Q system (Qiagen, Hilden, Germany) with gene-specific primer pairs (Eurofins MWG Operon, Ebersberg, Germany), as outlined in Chiappisi et al. (2017) and Keller et al. (2012). Primer details are provided in Table 2. To ensure the reliability of the data, qPCR results were normalized using the three most stable reference genes (CANX, MDH1, SDHA), based on the

TABLE 2 | Properties of primers for qPCR analysis.

Gene symbol	NCBI GenBank Accession Number	Primer sequence		Product size (bp)
		forward	reverse	
Reference genes				
ATP5F1B	NM_001686	TCGCGTGCCATTGCTGAGCT	CGTGCACGGGACACGGTCAA	218
SDHA	NM_004168	CCAAGCCCATCCAGGGGCAAC	TCCAGAGTGACCTTCCCAGTGCCAA	100
YWHAZ	NM_003406	TGGGGACTACGACGTCCCTCAA	CATATCGCTCAGCCTGCTCGG	115
Target genes				
FOXO3	NM_001455	CTACGAGTGGATGGTGCGTT	TGTGCCGGATGGAGTTCTTC	89
LMB1	NM_005573	AGGAGAAGGAGGAGCTGCG	GATTCCTTCTTAGCATAGTTGAGGA	286
STAT3	NM_139276	TCTGCCGGAGAAACAGTTGG	AGGTACCGTGTGTCAAGCTG	83
TP53	NM_000546	TGTGACTTGCACGTACTCCC	ACCATCGCTATCTGAGCAGC	199

methodology outlined by Vandesompele et al. (2002). Signal transducer and activator of transcription 3 (STAT3), Lamin B1 (LMB1), forkhead box O3 (FOXO3), and tumor protein P53 (TP52) were measured.

2.7 | Statistical Analysis

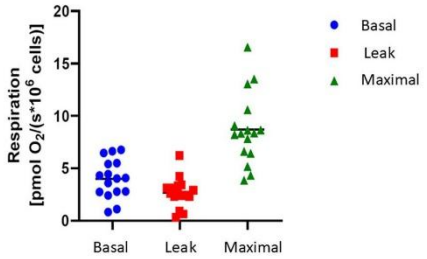
Given the exploratory nature of this study, a formal sample size calculation was not conducted. The sample size of 16 participants was determined based on feasibility and is comparable to previous studies examining mitochondrial function in PBMCs under similar conditions (Zhou et al. 2020). Data were analyzed using SPSS version 26 (IBM, Chicago, IL, USA) and GraphPad Prism (Windows, GraphPad Software, La Jolla, California, USA). Univariate linear regression analysis was performed to determine the associations between mitochondrial respiration (basal and maximal MR_{PBMC}) and various independent variables, including PBMC subpopulations, cytokine levels, intracellular signaling markers, and metabolic parameters. For continuous variables, regression coefficients (ß) and their 95% confidence interval (CI) were calculated, and statistical significance was set at $p < 0.05$. The strength of the associations between the variables was determined by evaluating the effect size, with higher ß values indicating stronger relationships. Descriptive statistics are presented as mean ± standard deviation (SD). All tests were two-tailed, and statistical significance was considered at $p < 0.05$. No adjustments for multiple comparisons were made, reflecting the exploratory nature of the study and its aim to identify broad associations rather than to test specific a priori hypotheses. Multivariate regression models were calculated to assess whether selected associations remained stable after accounting for potential confounders. These models are considered exploratory and serve to complement the univariate results. These models were constructed stepwise: first adjusting for age (Model 1), then sequentially including body fat percentage (Model 2) and VO_{2peak} as covariates to account for lifestyle-related influences (Model 3). To avoid overfitting, the number of predictors per model was limited, and only variables that were statistically significant or biologically plausible based on univariate results were considered.

3 | Results

3.1 | Associations Between MR_{PBMC} and PBMC Subpopulations

The main outcomes for mitochondrial respiration were basal respiration and maximal respiration (see Figure 1). LEAK respiration was not included in the regression analyses but was assessed as a control parameter to evaluate mitochondrial coupling efficiency and membrane integrity. To examine factors that influence MR_{PBMC} univariate linear regression analysis was performed to investigate PBMC subpopulations for their association with MR_{PBMC}. Univariate regression revealed associations between basal MR_{PBMC} and proportion of $CD4^+$ T cells (ß = 0.093 [0.013–0.173], $p < 0.05$), while maximal MR_{PBMC} was associated with percentage of $CD4^+$ (ß = 0.180 [0.033–0.328]), $CD8^+$ (0.263 [0.006–0.520]), CD4 naïve (ß = 0.121 [0.026–0.216]), and CD4EM cells (ß = −0.170 [−0.331–−0.009], all $p < 0.05$). No association was found between basal respiration of MR_{PBMC} and proportion of naïve $CD4^+$ cells, CD4EM, CD4CM, CD4TEMRA, all $CD8^+$ cells, T_{eff}, and all T_{regs} (all $p > 0.05$), and between maximal respiration of MR_{PBMC} and proportion of CD4CM, CD4TEMRA, naïve $CD8^+$, CD8EM, CD8CM, CD8TEMRA, T_{eff}, and all T_{regs} (all $p > 0.05$). More details are shown in Table 3.

A



B

	Oxygen concentration (µM)				Oxygen flow (pmol/(s*mL))			
	Basal	Leak	Maximal	ROX	Basal	Leak	Maximal	ROX
MW	195.95	192.56	194.76	175.36	3.99	2.67	8.69	1.88
SD	7.63	6.90	8.35	6.55	1.84	1.40	3.39	2.69

FIGURE 1 | (A) Basal, leak, and maximal respiration in the intact, unstimulated PBMCs. Mitochondrial respiration was measured as basal oxygen consumption in unstimulated PBMCs ("Basal"), after inhibition of complex V (ATPase) with oligomycin (proton leak respiration, "Leak"), and after stepwise titration with the uncoupler FCCP for maximal stimulation of the cells ("Maximal"). (B) Oxygen concentration and flow data in different states. All data are shown in mean ± SD; $n = 16$ participants were used.

19974652, 2025, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/sp.70096, Wiley Online Library on [01/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

10974652, 2025, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jcp.70096, Wiley Online Library on [01/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 3 | Univariate regression models for mitochondrial respiration of PBMCs and T cell subsets.

	Basal respiration			Maximal respiration		
Cell type	***Adjusted R²***	***ß (95% CI)***	***p* value**	***Adjusted R²***	***ß (95% CI)***	***p* value**
CD4	0.231	0.093 (0.013–0.173)	**0.025***	0.251	0.180 (0.033–0.328)	**0.020***
CD4 naive	−0.017	0.024 (−0.036–0.084)	0.409	0.271	0.121 (0.026–0.216)	**0.016***
CD4 EM	0.036	−0.057 (−0.150–0.037)	0.218	0.191	−0.170 (−0.331– −0.009)	**0.040***
CD4 CM	−0.055	−0.014 (−0.104–0.076)	0.746	0.016	−0.087 (−0.0250–0.076)	0.276
CD4TEMRA	−0.037	0.076 (−0.179–0.331)	0.536	0.033	−0.273 (−0.734–0.188)	0.228
CD8	0.062	0.101 (−0.046–0.247)	0.165	0.179	0.263 (0.006–0.520)	**0.046***
CD8 naive	−0.061	−0.005 (−0.077–0.067)	0.887	0.051	0.083 (−0.045–0.211)	0.187
CD8 EM	0.027	−0.063 (−0.173–0.047)	0.242	0.086	−0.152 (−0.352–0.048)	0.126
CD8 CM	0.124	−0.317 (−0.681–0.047)	0.084	−0.060	−0.063 (−0.814–0.687)	0.860
CD8TEMRA	−0.022	0.029 (−0.048–0.106)	0.438	0.019	0.077 (−0.064–0.219)	0.265
T_{eff}	−0.056	0.020 (−0.118–0.157)	0.763	−0.043	−0.065 (−0.321–0.191)	0.596
T_{reg}	−0.051	0.122 (−0.483–0.726)	0.675	−0.001	0.519 (−0.586–1.623)	0.334
T_{reg}/T_{eff} ratio	−0.050	8.367 (−32.188–48.922)	0.668	0.003	35.921 (−38.081–109.923)	0.319
mT_{reg}	−0.060	0.005 (−0.053–0.064)	0.850	−0.051	−0.021 (−0.130–0.088)	0.682
rT_{reg}	−0.039	−0.017 (−0.080–0.045)	0.560	−0.057	0.015 (−0.102–0.133)	0.786

Abbreviations: CM, central memory; EM, effector memory; mT_{reg}, memory-T_{reg}; T_{eff}, T effector cells; T_{reg}, regulatory T cells; rT_{reg}, resting-T_{reg}; 95% CI: 95% confidence interval.
*$p < 0.05$.

3.2 | Association Between MR_{PBMC}, Plasma Cytokine Levels, and Intracellular Signaling Molecules

Next, univariate regression analysis was performed to detect associations between MR_{PBMC} and levels of plasma cytokines. Absolute cytokine values are shown in Supporting Material Table S2. Results of regression analysis are shown in Table 4. Basal MR_{PBMC} was negatively associated with both IL-17 (ß = −0.879 [−1.659 to −0.100]) and TNF-α (ß = −0.730 [−1.426 to −0.034]) and positively associated with myoglobin (ß = 0.546 [0.016–1.075], all $p < 0.05$). Maximal MR_{PBMC} revealed positive associations to plasma levels of IL-8 (ß = 0.495 [0.130–0.860] $p = 0.01$), ICAM-1 (ß = 0.000026 [0.000006–0.000046], $p = 0.01$), and VEGF (ß = 0.027 [0.004–0.049], $p < 0.05$). For intracellular signaling, univariate linear regression was performed, and positive associations between basal respiration and level of STAT3 expression were found (ß = 5.910 [2.073–9.747], $p < 0.01$). No association was found between basal MR_{PBMC} and IL1ra, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, IFNγ, ICAM-1, VEGF, Adiponectin, Tenascin-C, Galectin, MMP2, MMP9, MPO, CCL4, CXCL9, CXCL13, GDF15, STAT3, LMB1, FOXO3, and TP53 (all $p > 0.05$), while no significant association was found between maximal MR_{PBMC} and IL1ra, IL-6, IL-7, IL-10, IL-15, IL-17, IL-18, TNF-α, IFNγ, Adiponectin, Tenascin-C, Galectin, MMP2, MMP9, MPO, Myoglobin, CCL4, CXCL9, CXCL13, GDF15, LMB1, FOXO3, and TP53 (all $p > 0.05$) (Table 5).

3.3 | Association of MRr_{PBMC} to Anthropometric Data, Cardiorespiratory Fitness, and Metabolic Risk Factors

Likewise, univariate linear regression was performed with MR_{PBMC} and anthropometric, fitness, and metabolic risk factors (see Table 6). Here, basal MR_{PBMC} was positively associated with age (ß = 0.260 [0.048–0.473], $p < 0.05$), levels of blood glucose (ß = 0.104 [0.0021–0.186], $p < 0.05$), while maximal MR_{PBMC} was associated with glucose (ß = 0.194 [0.039–0.348], $p < 0.05$) and HbA1c (ß = −5.777 [−11.277 to −0.277], $p < 0.05$). No association was found between basal MR_{PBMC} and BMI, body fat, VO_{2peak}, hand grip strength, and HbA1c (all $p > 0.05$), while no significant association was found between maximal MR_{PBMC} and age, BMI, body fat, VO_{2peak}, and hand grip strength (all $p > 0.05$).

3.4 | Exploratory Multivariate Regression Analyses

To further examine the robustness of the observed associations, we performed exploratory multivariate regression analyses adjusting stepwise for covariates. Detailed regression results for all models, including unstandardized coefficients, 95% CI, p-values, and adjusted R^2 values, are provided in Supporting Material Table S3. We first controlled for age alone, and subsequently added body fat % and VO_{2peak} to assess the potential influence of lifestyle-related factors. For basal MR_{PBMC}, the strongest model included $CD4^+$ T cell percentage and age (adjusted $R^2 = 0.436$, $p = 0.005$), with both variables showing significant associations ($CD4^+$: ß = 0.081, 95% CI [0.012, 0.150], $p = 0.025$; age: ß = 0.229, 95% CI [0.042, 0.417], $p = 0.020$). Similarly, TNF-α (ß = –0.703, 95% CI [–1.279, –0.127], $p = 0.020$) and age (ß = 0.253, 95% CI [0.069, 0.436], $p = 0.010$) were both independently associated in their model (adjusted $R^2 = 0.450$, $p = 0.004$). Age (ß = 0.207, 95% CI [0.000, 0.413], $p = 0.050$) also emerged as the sole significant predictor in the IL-17 model (adjusted $R^2 = 0.360$, $p = 0.014$), whereas IL-17 itself was not independently associated. In addition,

10974652, 2025, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jcp.70096, Wiley Online Library on [01/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 4 | Univariate regression models for mitochondrial respiration of PBMCs and cytokines.

	Basal respiration			Maximal respiration		
Molecule	**Adjusted R^2**	**ß (95% CI)**	***p* value**	**Adjusted R^2**	**ß (95% CI)**	***p* value**
IL1ra	0.039	−0.004 (−0.011–0.003)	0.213	−0.023	−0.005 (−0.019–0.009)	0.444
IL-6	−0.053	−0.069 (−0.403–0.264)	0.663	−0.065	−0.045 (0.−0.671–0.581)	0.880
IL-7	−0.004	−0.078 (−0.249–0.093)	0.349	−0.020	−0.125 (−0.448–0.199)	0.426
IL-8	0.016	0.123 (−0.108–0.354)	0.274	0.299	0.495 (0.130–0.860)	**0.011***
IL-10	−0.025	−0.686 (−2.589–1.218)	0.456	−0.059	−0.418 (−4.042–3.205)	0.810
IL-15	0.094	−0.173 (−0.393–0.048)	0.116	0.055	−0.280 (−0.702–0.141)	0.177
IL-17	0.217	−0.879 (−1.659 to −0.100)	**0.029***	0.138	−1.396 (−2.928–0.136)	0.071
IL-18	−0.060	0.00005 (−0.00044–0.00053.)	0.843	−0.061	−0.00006 (−0.00096–0.00085)	0.893
TNF-α	0.118	−0.730 (−1.426–−0.034)	**0.041***	0.017	−0.769 (−2.204–0.666)	0.273
IFNγ	0.153	−0.011 (−0.025–0.002)	0.083	0.136	−0.021 (−0.046–0.004)	0.097
ICAM-1	−0.014	0.000005 (−0.000007–0.000018)	0.397	0.282	0.000026 (0.000006–0.000046)	**0.014***
VEGF	0.003	0.007 (−0.007–0.020)	0.321	0.244	0.027 (0.004–0.049)	**0.022***
Adiponectin	−0.060	0.046 (−0.492–0.584)	0.858	−0.049	0.216 (−0.786–1.217)	0.654
Tenascin-C	−0.036	−0.364 (−1.579–0.850)	0.534	0.014	1.164 (−0.1055–3.383)	0.283
Galectin	−0.057	0.169 (−1.075–1.412)	0.777	−0.059	−0.234 (−2.566–2.097)	0.834
MMP2	−0.057	0.008 (−0.049–0.064)	0.779	0.004	0.050 (−0.053–0.153)	0.317
MMP9	0.061	−0.008 (−0.019–0.004)	0.116	0.033	−0.012 (−0.034–0.009)	0.228
MPO	0.148	−0.027 (−0.055–0.002)	0.064	−0.020	−0.022 (−0.081–0.036)	0.429
Myoglobin	0.182	0.546 (0.016–1.075)	**0.044**	0.054	0.706 (−0.360–1.771)	0.180
CCL4	0.086	−0.021 (−0.048–0.007)	0.127	0.033	−0.031 (−0.084–0.022)	0.227
CXCL9	0.015	−0.002 (−0.006–0.002)	0.279	0.075	−0.005 (−0.013–0.002)	0.142
CXCL13	−0.010	−0.021 (−0.069–0.028)	0.374	−0.062	−0.004 (−0.097–0.089)	0.933
GDF15	−0.013	0.00009 (−0.00013 to 0.00031)	0.389	0.017	0.00022 (−0.00019–0.00063)	0.271

Abbreviations: IL, interleukin; CCL4, carbon tetrachloride; CXCL, chemokine ligand; GDF15, growth/differentiation factor 15; ICAM1, intercellular adhesion molecule 1; MMP, matrix metallopeptidase; MPO, myeloperoxidase; TNF-α, tumour necrosis factor alpha; IFNγ, interferon-gamma; VEGF, vascular endothelial growth factor; 95% CI, 95% confidence interval.
*$p < 0.05$.

TABLE 5 | Univariate regression models for mitochondrial respiration of PBMCs and intracellular signaling markers.

	Basal respiration			Maximal respiration		
Gene	***Adjusted R^2***	***ß (95% CI)***	***p* value**	***Adjusted R^2***	***ß (95% CI)***	***p* value**
STAT3	−0.43	0.831 (−1.746–3.408)	0.493	0.466	5.910 (2.073–9.747)	**0.006***
LMB1	0.113	1.750 (−0.672–4.173)	0.140	0.042	2.945 (−2.296–8.185)	0.242
FOXO3	0.003	−0.724 (−2.293–0.845)	0.331	−0.085	0.382 (−3.024–3.789)	0.809
TP53	−0.099	−0.063 (−1.431–1.305)	0.920	−0.028	1.106 (−1.838–4.051)	0.422

Abbreviations: FOXO3, forkhead box O3; LMB1, Lamin B1; STAT3, signal transducer and activator of transcription 3; TP53, tumor protein P53; 95% CI, 95% confidence interval.
*$p < 0.05$.

fasting glucose remained significantly associated with basal MR_{PBMC} after full adjustment (ß = 0.092, 95% CI [0.007, 0.177], $p = 0.036$; adjusted R^2 = 0.373, $p = 0.037$). In contrast, for maximal mitochondrial respiration, $CD4^+$ T and naïve $CD4^+$ T cells showed the strongest univariate associations, which were not improved by adding lifestyle-related covariates. In contrast, $CD4^+$ effector memory T cells remained significantly associated in a multivariate model including age and body fat (ß = −0.200, 95% CI [−0.353, −0.046], $p = 0.015$; adjusted R^2 = 0.309, $p = 0.043$). IL-8 was significantly associated with basal MR_{PBMC}

10973652, 2025, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jcp.70096, Wiley Online Library on [01/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 6 | Univariate regression models for mitochondrial respiration of PBMCs and anthropometric data.

	Basal respiration			Maximal respiration		
Anthropometrics	***Adjusted R²***	***ß (95% CI)***	***p* value**	***Adjusted R²***	***ß (95% CI)***	***p* value**
Age	0.253	0.260 (0.048–0.473)	**0.019***	−0.022	0.175 (−0.290–0.641)	0.437
BMI	−0.047	0.074 (−0.254–0.402)	0.639	−0.044	0.156 (−0.457–0.769)	0.597
Body fat (%)	−0.030	−0.47 (−0.188–0.094)	0.491	0.001	0.123 (0.123)	0.329
VO_{2peak}	−0.029	0.034 (−0.067–0.136)	0.483	−0.060	−0.019 (−0.212–0.175)	0.841
Hand grip strength	0.030	0.023 (−0.017–0.063)	0.236	−0.055	0.012 (−0.090–0.066)	0.748
Glucose	0.264	0.104 (0.021–0.186)	**0.017***	0.263	0.194 (0.039–0.348)	**0.017***
HbA1c	−0.046	−0.784 (−4.119–2.551)	0.625	0.189	−5.777 (−11.277 to −0.277)	**0.041***

Abbreviation: 95% CI: 95% confidence interval.
*$p < 0.05$

(ß = 0.481, 95% CI [0.095, 0.867], $p = 0.018$), independently of age. Likewise, ICAM-1 and VEGF were the only independent predictors in their univariate models. $CD8^+$ T cells also showed independent explanatory value in the univariate model only. HbA1c was inversely associated with maximal MR_{PBMC} and remained the only significant predictor in the adjusted model 2 (ß = −6.865, 95% CI [−12.141, −1.589], $p = 0.014$; adjusted $R^2 = 0.310$, $p = 0.043$). Fasting glucose also showed a positive univariate association with maximal MR_{PBMC}, but this did not remain robust in multivariate models. Notably, the model including STAT3, age, body fat, and VO_{2peak} yielded the best overall fit for maximal MR_{PBMC} (adjusted $R^2 = 0.495$, $p = 0.047$), with STAT3 as the sole significant predictor (ß = 4.992, 95% CI [0.450, 9.534], $p = 0.035$). No significant contribution was observed for VO_{2peak} or body composition in any of the models.

4 | Discussion

Our results highlight how mitochondrial respiration of PBMCs is related to the proportion of $CD4^+$ T cell subsets, systemic inflammation, and activation of intracellular signaling pathways. In particular, our findings demonstrate that increased mitochondrial respiration is associated with both a more favorable distribution of $CD4^+$ T cell subtypes, especially naive T cells, and lower concentrations of pro-inflammatory cytokines such as TNF-α and IL-17. Furthermore, the observed positive correlations between maximal mitochondrial respiration and vascular markers such as ICAM-1 and VEGF demonstrate how mitochondrial bioenergetics might affect both immune and vascular function. These findings emphasize the central role of mitochondrial function as a potential key factor in promoting healthy aging.

Age-associated changes in the functionality of immune cells, particularly in subtypes of PBMCs, and their link to chronic inflammation and decreased immunity has already been researched from different perspectives, but the metabolic consequences for mitochondrial respiration remained unclear (Miwa et al. 2022). In this healthy elderly cohort, the whole $CD4^+$ T cell pool was positively associated with basal and maximal MR_{PBMC}. Herein, a higher percentage of naïve $CD4^+$ T cells was associated with an increased maximal MR_{PBMC}. These associations remained robust after adjusting for age, body fat, and VO_{2peak}. These findings are consistent with data from other studies, which demonstrate that $CD4^+$ T cells from older adults exhibit elevated basal and maximal oxygen consumption rates (Bharath et al. 2020). Previous data by Withnall et al. (2024) demonstrated a higher mitochondrial dependence of $CD4^+$ T cells, naïve $CD8^+$, EM, and CM T cells in older adults, whereas $CD4^+$ T cells in younger adults showed a greater reliance on glycolytic metabolism. The proportion of naïve $CD4^+$ T cells tends to decrease within aging, while effector and memory cells increase (Boßlau et al. 2021). Therefore, only a change in the composition of the PBMC subpopulations due to differences in metabolic performance can influence overall respiratory performance (Hodgman et al. 2023). Nevertheless, there is also evidence of impaired mitochondrial respiration, including basal and maximal respiration in $CD4^+$ T cells in older adults (Bektas et al. 2019). In this regard, an accumulation of mitochondrial proteins in $CD4^+$ T cells from older adults was found compared to younger adults suggesting a lack of mitochondrial recycling due to defective autophagy (Bektas et al. 2019; Sriwichaiin et al. 2023). In addition, $CD8^+$ T cells also undergo age-related changes in both their composition and function. As individuals age, the number of naïve $CD8^+$ T cells decreases, while the proportion of memory and effector cells increases (Ponnappan and Ponnappan 2011). These shifts not only impact the immune response but also influence the metabolic profile of $CD8^+$ T cells.

In our study, the percentage of $CD4^+$EM cells was associated with lower maximal MR_{PBMC}. Even after accounting for age and body fat in multivariate models, $CD4^+$EM T cells remained independently associated with reduced maximal respiration. This is contrasted by findings that in adults of different age, memory $CD4^+$ T cells had higher oxidative phosphorylation than naïve $CD4^+$ T cells due to the increase of CPT1a expression. Following a polyclonal stimulation, $CD4^+$ memory T cells from older adults demonstrated increased mitochondrial activity (OXPHOS), including higher ROS and ATP levels, independent of activation-induced mitochondrial biogenesis. While those aged $CD4^+$ memory T cells maintained a more catabolic lipid metabolism, these cells retained the ability to enhance glycolysis upon activation (Yanes et al. 2019).

10974652, 2025, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jsp2.70096, Wiley Online Library on [01/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Aging displays a state of chronic low-grade inflammation with increased serum levels of inflammatory markers. Research in elderly people has shown a decreased frequency of IL-17-producing cells in the memory subset of $CD4^+$ T cells compared to younger people (Coperchini et al. 2024). In general, levels of IL-17 released by various immune cells such as NK cells or neutrophils increase with age and in particular $CD4^+$ T cells from healthy older people preferentially develop a Th17 profile (Bharath et al. 2020; Xu et al. 2024). In our healthy elderly cohort, IL-17 was inversely associated with basal mitochondrial respiration in univariate analyses; however, this relationship did not persist after adjusting for age. This suggests that the observed association likely reflects age-related immunological shifts rather than a direct metabolic link. Consequently, IL-17 may serve more as a marker of immune system remodeling than as a determinant of mitochondrial function in this population (Huangfu et al. 2023). Potential mechanisms, such as suppressed glycolysis—relevant for IL-17 production—or enhanced mitochondrial resilience through reduced inflammatory signaling, remain speculative in this context.

The observed relationship between inflammation and mitochondrial respiration further highlights the role of TNF-α, a key cytokine for overall inflammatory responses, whose signaling pathway activation represents a biomarker of cellular senescence (Bao et al. 2023). Within our healthy aging cohort, serum TNF-α was negatively associated with the basal MR_{PBMC}. This association persisted after adjustment for age, supporting the role of TNF-α as an independent marker of reduced mitochondrial function. TNF-α drives inflammation and ensures immune cells' activation following a change in cellular energy metabolism (Bao et al. 2023). However, the data from the literature are very heterogeneous. Various studies show that TNF-α is chronically elevated to a slight degree in old age (Brüünsgaard and Pedersen 2003; P. Li et al. 2015). In early-stage heart failure patients, a negative correlation between the mitochondrial respiratory function and TNF-α, IL-6, CRP, and cardiometabolic risk factors was found, while the basal and maximal MR_{PBMC} was reduced compared to a healthy control group (P. Li et al. 2015). Although the role of TNF-α in the aging process continues to be widely discussed, our results suggest that improved mitochondrial function–recognizable by higher basal MR_{PBMC}–could attenuate inflammatory processes. This is supported by the lower TNF-α serum levels observed in our cohort. However, we can only show a connection, not causality. Accordingly, it is also conceivable that TNF-α conversely inhibits oxidative metabolism.

Cellular senescence is a central mechanism for the development of cardiovascular and atherosclerotic diseases due to disturbed vascular endothelial function (Hu et al. 2022). ICAM-1 is expressed by the vascular endothelium, monocytes/macrophages, and lymphocytes and is demonstrated to be increased in both, healthy older adults and patients with pathological diseases, including hypertension, diabetes, and atherosclerosis (Dougherty et al. 1988; Van der Meer et al. 2002). In our healthy, older study cohort, we found a positive association between maximal MR_{PBMC} and ICAM-1 serum levels independent of lifestyle covariates. One the one hand, an increased maximal MR_{PBMC} may serve as a mechanism to counteract oxidative stress or age-related inflammatory processes, on the other hand, a higher mitochondrial capacity might reflect an improved ability to support immune responses through i.e. ICAM-1-mediated cellular dynamics.

In addition, intracellular signaling showed associations with mitochondrial respiration, in our study with STAT signaling. Within aging, a relation to progressively higher levels of STAT3 signaling activity was previously found in PBMCs of older adults. Here, higher STAT3 was suggested to be driven by age-related increases of systemic inflammation (Piber et al. 2019). Furthermore, there is evidence that aging promotes localization of STAT3 in the mitochondria of $CD4^+$ T cells, leading to changes in the structure and function of mitochondria, which affects mitochondrial dynamics and cytokine production (Zukowski et al. 2023). The positive correlation between maximal MR_{PBMC} and STAT3 suggests that STAT3 may enhance mitochondrial capacity either directly within mitochondria or indirectly via transcriptional regulation. Among all variables tested, STAT3 exhibited the strongest and most consistent association with maximal mitochondrial respiration, independent of all covariates. This highlights its key role in linking cellular energy metabolism and immune function. Similarly, VEGF and IL-8 exhibit notable connections to mitochondrial respiration and inflammation. VEGF, known for its key function as a growth factor in angiogenesis, vascular maintenance, and inflammation, has been shown to enhance mitochondrial function in endothelial cells and mitochondria play a dominant role in the stimulation of VEGF (D. Guo et al. 2017).

Our findings reveal a positive association between maximal MR_{PMBC}, serum VEGF, and serum IL-8 levels. In multivariate analyses, both VEGF and IL-8 remained independently associated with maximal respiration. IL-8, a potent, proangiogenic, and inflammatory chemokine, has been shown to upregulate VEGF expression in endothelial cells (Martin et al. 2009). The mechanistic role of STAT3 in enhancing mitochondrial capacity may underpin this relationship, as greater mitochondrial efficiency could meet the energy demands of processes mediated by VEGF and IL-8. VEGF's association with MR_{PBMC} may indicate its involvement not only in oxygen delivery but also in supporting cellular energy needs, potentially optimizing PBMC function under mitochondrial stress. Similarly, enhanced mitochondrial capacity could improve PBMC responsiveness to IL-8 signaling, particularly during inflammatory challenges. These associations might reflect improved vascularization, immune competence, and enhanced overall fitness in a healthy aging cohort. However, the absence of correlations between MR_{PBMC} and fitness parameters, despite VO_{2peak} values aligning with ACSM standards, may stem from the cohort's homogeneity and lack of disease burden (Riebe et al. 2018). This is further supported by the lack of independent associations between VO_{2peak} and mitochondrial respiration in any of the multivariate models.

A healthy and active lifestyle has been a key research focus in recent decades, particularly regarding its impact on mitochondrial respiration in immune cells. Cardiorespiratory fitness (CRF), typically assessed as VO_{2peak}, has been shown to influence mitochondrial respiration in PBMCs, altering their transcriptomic and metabolic profile (Gebhardt et al. 2024). Various studies showed that interventions including physical activity

and exercise over weeks are followed by mitochondrial capacity changes towards a higher maximal respiration (Krüger and Gebhardt 2024). Studies on active older adults and lifelong exercisers suggest a reduced inflammatory state compared to their sedentary counterparts, potentially mediated by enhanced mitochondrial function (Pérez-Castillo et al. 2025). In our healthy aging cohort, basal MR_{PBMC} showed a positive association with age, supporting the hypothesis of increased mitochondrial dependence in aging. However, the relationship between mitochondrial respiration and age remains inconclusive due to heterogeneous findings in the literature (Ehinger et al. 2024).

Aging is also associated with metabolic changes that can affect glucose homeostasis. Impairments in insulin sensitivity and glucose regulation are well-documented in aging populations, often linked to alterations in mitochondrial function. A possible connection between these processes is reflected in our findings. The negative association between MR_{PBMC} and HbA1c levels could indicate metabolic dysfunction in the context of impaired glucose homeostasis. There is also evidence for this in PBMCs, which has already been linked to altered gene expression in those, particularly in genes involved in cell cycle processes (Slieker et al. 2018).

These findings point to mitochondrial respiration in PBMCs as a potential target for future interventions aimed at supporting immune resilience in aging. Approaches such as physical activity may modulate mitochondrial bioenergetics and thereby contribute to a more balanced immune phenotype, but this requires validation in longitudinal or interventional studies. However, given the exploratory character of this study, several limitations must be considered when interpreting the results. First, the small sample size limits statistical power, increasing the risk of Type II errors, particularly for small to moderate associations. Second, multiple univariate analyses were performed without adjustments for multiple comparisons, reflecting the exploratory nature of the study aimed at identifying broad patterns rather than testing predefined hypotheses. Third, although exploratory multivariate models were used to adjust for potential confounders of main findings, their results should be interpreted cautiously. Consequently, findings should be seen as hypothesis-generating and require validation in larger cohorts. Fourth, subgroup sizes were too small for stratified analyses by sex. Future studies with sufficient power are needed to address potential sex-specific differences. Fifth, the healthy cohort, consisting of participants free of diagnosed diseases and medications, differs from many prior studies that included individuals with pre-existing conditions or treatments that could influence immune or mitochondrial function. Finally, the cross-sectional design limits causal inferences, as observed associations reflect correlations at a single time point. Additionally, while some studies report that cryopreservation time does not adversely affect mitochondrial function, others suggest that samples should ideally be analyzed within a week post-freezing due to declining respiratory capacities (Dieter et al. 2023; Werner et al. 2022). More recently, Payne et al. (2025) demonstrated that mitochondrial function can be preserved for up to 3 months at −80°C, but since our PBMCs were stored for up to 6 months, potential alterations due to prolonged cryopreservation cannot be excluded. It is important to note that we did not assessed mitochondrial mass, so our findings represent cell-based respiratory capacity rather than mitochondria-specific function. This distinction is critical, as differences in respiration profiles between groups may reflect variations in mitochondrial content rather than solely cell-based capacity, and such respiration rates may mask underlying deficits unless normalized to mtDNA copy number (Alonso et al. 2021; Sartori et al. 2025). Future studies with larger and more diverse cohorts, applying longitudinal designs and adequately powered multivariate analyses, are needed to validate and extend these findings. They should include normalization per mitochondrion to enable more precise interpretation of mitochondrial function.

5 | Conclusion

In conclusion, this study highlights significant associations between mitochondrial respiration in PBMCs and key immunological, inflammatory, and metabolic markers in a healthy elderly cohort. Notably, higher mitochondrial respiration was positively associated with a more favorable distribution of $CD4^+$ T cell subsets, particularly naïve T cells, which are indicative of robust immune function. Conversely, elevated levels of pro-inflammatory cytokines, such as TNF-α were inversely associated with basal mitochondrial respiration, suggesting a potential link between chronic low-grade inflammation and impaired mitochondrial function during aging. These findings emphasize the importance of mitochondrial function as a critical determinant of immunometabolic health in aging populations. Future studies should further investigate the mechanistic pathways linking mitochondrial bioenergetics, immune cell metabolism, and healthy aging, while exploring interventions - such as physical activity and targeted lifestyle modifications - that could preserve mitochondrial function and promote successful aging.

Author Contributions

Kristina Gebhardt: conceptualization, development and design of methodology, investigation, formal analysis, writing- original draft, visualization, writing – review and editing. **Anne Hebecker:** investigation. **Magdalena Huber:** conceptualization, methodology, funding acquisition. **Hartmann Raifer:** methodology, validation, investigation. **Natascha Sommer:** conceptualization, methodology, funding acquisition. **Robert Ringseis:** conceptualization, methodology, validation, investigation, writing – review and editing, funding acquisition. **Klaus Eder:** conceptualization, writing – review and editing, funding acquisition. **Karsten Krüger:** conceptualization, methodology, writing – original draft, writing – review and editing, supervision, funding acquisition. **Christopher Weyh:** conceptualization, methodology, validation, investigation, formal analysis, writing – original draft, writing – review and editing.

Acknowledgments

We would like to thank the Forschungscampus Mittelhessen for funding the project. This study was funded by the Forschungscampus Mittelhessen, Flexi Fund Project number 20121_1_1_10. Open Access funding enabled and organized by Projekt DEAL.

Ethics Statement

The study was approved by the Medical Ethics Committee of Justus-Liebig University Giessen (AZ 100/20). All procedures were conducted in line with the Declaration of Helsinki.

10974652, 2025, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jcp.70096, Wiley Online Library on [01/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

10974652, 2025, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jcp.70096, Wiley Online Library on [01/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Consent

Written informed consent was obtained from all participants before their inclusion in the study.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

References

Almeida, L., M. Lochner, L. Berod, and T. Sparwasser. 2016. "Metabolic Pathways in T Cell Activation and Lineage Differentiation." *Seminars in Immunology* 28, no. 5: 514–524. https://doi.org/10.1016/j.smim.2016.10.009.

Alonso, M., C. Zabala, S. Mansilla, et al. 2021. "Blood Cell Respiration Rates and Mtdna Copy Number: A Promising Tool for the Diagnosis of Mitochondrial Disease." *Mitochondrion* 61: 31–43. https://doi.org/10.1016/j.mito.2021.09.004.

Avram, V. F., A. P. Merce, I. M. Hâncu, et al. 2022. "Impairment of Mitochondrial Respiration in Metabolic Diseases: An Overview." *International Journal of Molecular Sciences* 23, no. 16: 8852. https://doi.org/10.3390/ijms23168852.

Bao, H., J. Cao, M. Chen, et al. 2023. "Biomarkers of Aging." *Science China. Life Sciences* 66, no. 5: 893–1066. https://doi.org/10.1007/s11427-023-2305-0.

Bektas, A., S. H. Schurman, M. Gonzalez-Freire, et al. 2019. "Age-Associated Changes in Human CD4+ T Cells Point to Mitochondrial Dysfunction Consequent to Impaired Autophagy." *Aging* 11, no. 21: 9234–9263. https://doi.org/10.18632/aging.102438.

Bharath, L. P., M. Agrawal, G. McCambridge, et al. 2020. "Metformin Enhances Autophagy and Normalizes Mitochondrial Function to Alleviate Aging-Associated Inflammation." *Cell Metabolism* 32, no. 1: 44–55.e6. https://doi.org/10.1016/j.cmet.2020.04.015.

Böttrich, T., P. Bauer, V. Größer, et al. 2024. "Subpopulations of Regulatory T Cells Are Associated With Subclinical Atherosclerotic Plaques, Levels of LDL, and Cardiorespiratory Fitness in the Elderly." *Journal of Sport and Health Science* 13, no. 3: 288–296. https://doi.org/10.1016/j.jshs.2023.11.004.

Boßlau, T. K., P. Wasserfurth, B. Krüger, et al. 2021. "Abdominal Obesity-Related Disturbance of Insulin Sensitivity Is Associated With CD8+ EMRA Cells in the Elderly." *Cells* 10, no. 5: 998. https://doi.org/10.3390/cells10050998.

Brüünsgaard, H., and B. K. Pedersen. 2003. "Age-Related Inflammatory Cytokines and Disease." *Immunology and Allergy Clinics of North America* 23, no. 1: 15–39. https://doi.org/10.1016/s0889-8561(02)00056-5.

Chen, M., Y. Wang, S. Deng, Z. Lian, and K. Yu. 2022. "Skeletal Muscle Oxidative Stress and Inflammation in Aging: Focus on Antioxidant and Anti-Inflammatory Therapy." *Frontiers in Cell and Developmental Biology* 10: 964130. https://doi.org/10.3389/fcell.2022.964130.

Chiappisi, E., R. Ringseis, K. Eder, and D. K. Gessner. 2017. "Effect of Endoplasmic Reticulum Stress on Metabolic and Stress Signaling and Kidney-Specific Functions in Madin-Darby Bovine Kidney Cells." *Journal of Dairy Science* 100, no. 8: 6689–6706. https://doi.org/10.3168/jds.2016-12406.

Conte, M., M. Martucci, A. Chiariello, C. Franceschi, and S. Salvioli. 2020. "Mitochondria, Immunosenescence and Inflammaging: A Role for Mitokines?" *Seminars in Immunopathology* 42, no. 5: 607–617. https://doi.org/10.1007/s00281-020-00813-0.

Coperchini, F., A. Greco, M. Teliti, et al. 2024. "Inflamm-Ageing: How Cytokines and Nutrition Shape the Trajectory of Ageing." *Cytokine & Growth Factor Reviews* 82: 31–42. https://doi.org/10.1016/j.cytogfr.2024.08.004.

Dieter, F., J. Grube, T. Birkenhauer, A. Quentin, and G. P. Eckert. 2023. "Cryopreservation and Bioenergetic Evaluation of Human Peripheral Blood Mononuclear Cells." *Journal of Visualized Experiments* 200: e65730. https://doi.org/10.3791/65730.

Divakaruni, A. S., and M. Jastroch. 2022. "A Practical Guide for the Analysis, Standardization and Interpretation of Oxygen Consumption Measurements." *Nature Metabolism* 4, no. 8: 978–994. https://doi.org/10.1038/s42255-022-00619-4.

Dougherty, G. J., S. Murdoch, and N. Hogg. 1988. "The Function of Human Intercellular Adhesion molecule-1 (ICAM-1) in the Generation of An Immune Response." *European Journal of Immunology* 18, no. 1: 35–39. https://doi.org/10.1002/eji.1830180107.

Ehinger, J. K., E. Westerlund, E. Å. Frostner, et al. 2024. "Mitochondrial Function in Peripheral Blood Cells Across the Human Lifespan." *Npj Aging* 10, no. 1: 10. https://doi.org/10.1038/s41514-023-00130-4.

Gebhardt, K., A. Hebecker, C. Honekamp, et al. 2024. "Respiratory and Metabolic Responses of CD4 + T Cells to Acute Exercise and Their Association With Cardiorespiratory Fitness." *Medicine & Science in Sports & Exercise* 56, no. 10: 1882–1892. https://doi.org/10.1249/MSS.0000000000003483.

Guo, D., Q. Wang, C. Li, Y. Wang, and X. Chen. 2017. "Vegf Stimulated the Angiogenesis by Promoting the Mitochondrial Functions." *Oncotarget* 8, no. 44: 77020–77027. https://doi.org/10.18632/oncotarget.20331.

Guo, J., X. Huang, L. Dou, et al. 2022. "Aging and Aging-Related Diseases: From Molecular Mechanisms to Interventions and Treatments." *Signal Transduction and Targeted Therapy* 7, no. 1: 391. https://doi.org/10.1038/s41392-022-01251-0.

Harrington, J. S., S. W. Ryter, M. Plataki, D. R. Price, and A. M. K. Choi. 2023. "Mitochondria in Health, Disease, and Aging." *Physiological Reviews* 103, no. 4: 2349–2422. https://doi.org/10.1152/physrev.00058.2021.

Hedges, C. P., J. S. T. Woodhead, H. W. Wang, et al. 2019. "Peripheral Blood Mononuclear Cells Do Not Reflect Skeletal Muscle Mitochondrial Function or Adaptation to High-Intensity Interval Training in Healthy Young Men." *Journal of Applied Physiology* 126, no. 2: 454–461. https://doi.org/10.1152/japplphysiol.00777.2018.

Hodgman, C. F., R. M. Hunt, J. C. Crane, M. T. Elzayat, and E. C. LaVoy. 2023. "A Scoping Review on the Effects of Physical Exercise and Fitness on Peripheral Leukocyte Energy Metabolism in Humans." *Exercise Immunology Review* 29: 54–87.

Hu, C., X. Zhang, T. Teng, Z. G. Ma, and Q. Z. Tang. 2022. "Cellular Senescence in Cardiovascular Diseases: A Systematic Review." *Aging and Disease* 13, no. 1: 103–128. https://doi.org/10.14336/AD.2021.0927.

Huangfu, L., R. Li, Y. Huang, and S. Wang. 2023. "The IL-17 Family in Diseases: From Bench to Bedside." *Signal Transduction and Targeted Therapy* 8, no. 1: 402. https://doi.org/10.1038/s41392-023-01620-3.

Keller, J., R. Ringseis, A. Koc, I. Lukas, H. Kluge, and K. Eder. 2012. "Supplementation With L-Carnitine Downregulates Genes of the Ubiquitin Proteasome System in the Skeletal Muscle and Liver of Piglets." *Animal* 6, no. 1: 70–78. https://doi.org/10.1017/S1751731111001327.

Krüger, K., and K. Gebhardt. 2024. "Effects of Cardiorespiratory Fitness on Immune Cell Mitochondrial Metabolism in Health and Disease." *Deutsche Zeitschrift Für Sportmedizin* 75, no. 4: 123–131. https://doi.org/10.5960/dzsm.2024.587.

Li, P., B. Wang, F. Sun, et al. 2015. "Mitochondrial Respiratory Dysfunctions of Blood Mononuclear Cells Link With Cardiac Disturbance in Patients With Early-Stage Heart Failure." *Scientific Reports* 5: 10229. https://doi.org/10.1038/srep10229.

Li, X., C. Li, W. Zhang, Y. Wang, P. Qian, and H. Huang. 2023. "Inflammation and Aging: Signaling Pathways and Intervention Therapies." *Signal Transduction and Targeted Therapy* 8, no. 1: 239. https://doi.org/10.1038/s41392-023-01502-8.

Li, Y., X. Tian, J. Luo, T. Bao, S. Wang, and X. Wu. 2024. "Molecular Mechanisms of Aging and Anti-Aging Strategies." *Cell Communication and Signaling* 22, no. 1: 285. https://doi.org/10.1186/s12964-024-01663-1.

Martin, D., R. Galisteo, and J. S. Gutkind. 2009. "CXCL8/IL8 Stimulates Vascular Endothelial Growth Factor (VEGF) Expression and the Autocrine Activation of VEGFR2 in Endothelial Cells by Activating NFκB Through the CBM (Carma3/Bcl10/Malt1) Complex." *Journal of Biological Chemistry* 284, no. 10: 6038–6042. https://doi.org/10.1074/jbc.C800207200.

Van der Meer, I. M., M. P. M. de Maat, M. L. Bots, et al. 2002. "Inflammatory Mediators and Cell Adhesion Molecules as Indicators of Severity of Atherosclerosis: The Rotterdam Study." *Arteriosclerosis, Thrombosis, and Vascular Biology* 22, no. 5: 838–842. https://doi.org/10.1161/01.atv.0000016249.96529.b8.

Memme, J. M., A. T. Erlich, G. Phukan, and D. A. Hood. 2021. "Exercise and Mitochondrial Health." *Journal of Physiology* 599, no. 3: 803–817. https://doi.org/10.1113/JP278853.

Menshikova, E. V., V. B. Ritov, L. Fairfull, R. E. Ferrell, D. E. Kelley, and B. H. Goodpaster. 2006. "Effects of Exercise on Mitochondrial Content and Function in Aging Human Skeletal Muscle." *Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 61, no. 6: 534–540. https://doi.org/10.1093/gerona/61.6.534.

Miwa, S., S. Kashyap, E. Chini, and T. von Zglinicki. 2022. "Mitochondrial Dysfunction in Cell Senescence and Aging." *Journal of Clinical Investigation* 132, no. 13: 1–9. https://doi.org/10.1172/JCI158447.

O'Neill, L. A. J., R. J. Kishton, and J. Rathmell. 2016. "A Guide to Immunometabolism for Immunologists." *Nature Reviews Immunology* 16, no. 9: 553–565. https://doi.org/10.1038/nri.2016.70.

Payne, C., E. Louw, N. Baines, et al. 2025. "Mitochondrial Respiratory Analysis of Cryopreserved Pbmcs Isolated From Human Blood." *Cryobiology* 118: 105212. https://doi.org/10.1016/j.cryobiol.2025.105212.

Pérez-Castillo, I. M., R. Rueda, H. Bouzamondo, et al. 2025. "Does Lifelong Exercise Counteract Low-Grade Inflammation Associated With Aging? A Systematic Review and Meta-Analysis." *Sports Medicine* 55: 675–696. https://doi.org/10.1007/s40279-024-02152-8.

Piber, D., R. Olmstead, J. H. Cho, et al. 2019. "Inflammaging: Age and Systemic, Cellular, and Nuclear Inflammatory Biology in Older Adults." *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 74, no. 11: 1716–1724. https://doi.org/10.1093/gerona/glz130.

Ponnappan, S., and U. Ponnappan. 2011. "Aging and Immune Function: Molecular Mechanisms to Interventions." *Antioxidants & Redox Signaling* 14, no. 8: 1551–1585. https://doi.org/10.1089/ars.2010.3228.

Riebe, D., Ehrman, J. K., Liguori, G., and Magal, M., ed. 2018. *Acsm's Guidelines for Exercise Testing and Prescription*, 10th ed. Wolters Kluwer.

Sartori, L. F., E. Tsemberis, T. Hernandez, et al. 2025. "Distinct Mitochondrial Respiration Profiles in Pediatric Patients With Febrile Illness Versus Sepsis." *Pediatric Research* 97, no. 3: 1127–1133. https://doi.org/10.1038/s41390-024-03420-z.

Slieker, R. C., A. A. W. A. van der Heijden, N. van Leeuwen, et al. 2018. "Hba1c Is Associated With Altered Expression in Blood of Cell Cycle- and Immune Response-Related Genes." *Diabetologia* 61, no. 1: 138–146. https://doi.org/10.1007/s00125-017-4467-0.

Sommer, N., F. F. Theine, O. Pak, et al. 2022. "Mitochondrial Respiration in Peripheral Blood Mononuclear Cells Negatively Correlates With Disease Severity in Pulmonary Arterial Hypertension." *Journal of Clinical Medicine* 11, no. 14: 4132. https://doi.org/10.3390/jcm11144132.

Sriwichaiin, S., N. Apaijai, A. Phrommintikul, et al. 2023. "Increased Efficiency of Mitochondrial Coupling With a Reduction in Other Mitochondrial Respiratory Parameters in Peripheral Blood Mononuclear Cells Is Observed in Older Adults." *Journals of Gerontology: Series A* 78, no. 3: 384–391. https://doi.org/10.1093/gerona/glac201.

Tonkonogi, M., B. Walsh, M. Svensson, and K. Sahlin. 2000. "Mitochondrial Function and Antioxidative Defence in Human Muscle: Effects of Endurance Training and Oxidative Stress." *Journal of Physiology* 528, no. Pt 2: 379–388. https://doi.org/10.1111/j.1469-7793.2000.00379.x.

Tyrrell, D. J., M. S. Bharadwaj, C. G. van Horn, A. P. Marsh, B. J. Nicklas, and A. J. A. Molina. 2015. "Blood-Cell Bioenergetics Are Associated With Physical Function and Inflammation in Overweight/Obese Older Adults." *Experimental Gerontology* 70: 84–91. https://doi.org/10.1016/j.exger.2015.07.015.

Vandesompele, J., K. De Preter, F. Pattyn, et al. 2002. "Accurate Normalization of Real-Time Quantitative Rt-Pcr Data by Geometric Averaging of Multiple Internal Control Genes." *Genome Biology* 3, no. 7: research0034.1. https://doi.org/10.1186/gb-2002-3-7-research0034.

Werner, B. A., P. J. McCarty, A. L. Lane, et al. 2022. "Time Dependent Changes in the Bioenergetics of Peripheral Blood Mononuclear Cells: Processing Time, Collection Tubes and Cryopreservation Effects." *American Journal of Translational Research* 14, no. 3: 1628–1639.

Withnall, E., J. Hazeldine, A. Llibre, N. A. Duggal, J. M. Lord, and A. V. Sardeli (2024). *Physical Activity Modifies the Metabolic Profile of CD4+ and CD8+ T Cell Subtypes at Rest and Upon Activation in Older Adults*. https://doi.org/10.1101/2024.08.20.607078.

Xu, Y. Y., Z. Wang, S. Li, et al. 2024. "An In-Depth Understanding of the Role and Mechanisms of T Cells in Immune Organ Aging and Age-Related Diseases." *Science China. Life Sciences* 68: 328–353. https://doi.org/10.1007/s11427-024-2695-x.

Yanes, R. E., H. Zhang, Y. Shen, C. M. Weyand, and J. J. Goronzy. 2019. "Metabolic Reprogramming in Memory CD4 T Cell Responses of Old Adults." *Clinical Immunology* 207: 58–67. https://doi.org/10.1016/j.clim.2019.07.003.

Yang, J., J. Luo, X. Tian, Y. Y. Zhao, Y. Li, and X. Wu. 2024. "Progress in Understanding Oxidative Stress, Aging, and Aging-Related Diseases." *Antioxidants* 13, no. 4: 394. https://doi.org/10.3390/antiox13040394.

Yu, S. B., and G. Pekkurnaz. 2018. "Mechanisms Orchestrating Mitochondrial Dynamics for Energy Homeostasis." *Journal of Molecular Biology* 430, no. 21: 3922–3941. https://doi.org/10.1016/j.jmb.2018.07.027.

Zhou, B., D. D. H. Wang, Y. Qiu, et al. 2020. "Boosting Nad Level Suppresses Inflammatory Activation of Pbmcs in Heart Failure." *Journal of Clinical Investigation* 130, no. 11: 6054–6063. https://doi.org/10.1172/JCI138538.

Zukowski, E., M. Sannella, J. D. Rockhold, et al. 2023. "Stat3 Modulates CD4+ T Mitochondrial Dynamics and Function in Aging." *Aging cell* 22, no. 11: e13996. https://doi.org/10.1111/acel.13996.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.
Figure S1: Typical trace from intact peripheral blood mononuclear cells (PBMCs) from older participants using Oxygraph-2k. **Table S1:** Cytokine ranges and Detection rates. Table S2. Absolute values of serum cytokines. Table S3: Multivariate regression model.

10974652, 2025, 9, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jcp.70096, Wiley Online Library on [01/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

5.3 Effects of Cardiorespiratory Fitness on Immune Cell Mitochondrial Metabolism in Health and Disease

Gebhardt K and Krüger K (2024). Effects of Cardiorespiratory Fitness on Immune Cell Mitochondrial Metabolism in Health and Disease. Dtsch Z Sportmed. 2024; 75:123-131.

DOI: https://doi.org/10.5960/dzsm.2024.587

Supplementary Material: https://www.germanjournalsportsmedicine.com/archive/archive-2024/issue-4/effects-of-cardiorespiratory-fitness-on-immune-cell-mitochondrial-metabolism-in-health-and-disease/

Specification of the authors' contributions:
Conceptualization: Kristina Gebhardt; **Development and design of methodology:** Kristina Gebhardt**; Investigation:** Kristina Gebhardt; **Formal analysis:** Kristina Gebhardt; **Writing – Original Draft:** Kristina Gebhardt, Karsten Krüger; **Visualization:** Kristina Gebhardt; **Writing – review & editing:** Kristina Gebhardt, Karsten Krüger; **Funding acquisition:** Karsten Krüger

Gebhardt K[1], Krüger K[1]

Effects of Cardiorespiratory Fitness on Immune Cell Mitochondrial Metabolism in Health and Disease

Effekte der kardiorespiratorischen Fitness auf den mitochondrialen Immunzellmetabolismus in Gesundheit und Krankheit

REVIEW

ACCEPTED: January 2024

PUBLISHED ONLINE: June 2024

Gebhardt K, Krüger K. Effects of cardiorespiratory fitness on immune cell mitochondrial metabolism in health and disease. Dtsch Z Sportmed. 2024; 75: 123-131. doi:10.5960/dzsm.2024.587

1. JUSTUS LIEBIG UNIVERSITY GIESSEN, *Institute of Sports Science, Department of Exercise Physiology and Sports Therapy, Giessen, Germany*

Summary

- **Problem:** Cardiorespiratory fitness (CRF) and mitochondrial function are important factors in health and disease. Even in immune cells, an effective metabolism is crucial for most major cellular functions, such as T-cell proliferation. Little is known about the relationship between CRF and mitochondrial metabolism of immune cells.
- **Methods:** In this narrative review, we collected the most recent literature in the field of exercise immunology and mitochondrial immune cell metabolism. We included studies, which used Oxygraph- or Seahorse techniques for measurement of mitochondrial function in various immune cell populations and subtypes associated with topics in the field of exercise in health and disease.
- **Results:** 20 studies were included in our analysis. Cell populations were PBMCs (peripheral blood mononuclear **cells), lymphocytes, T cells, NK cells, platelets, and neutrophils. While mitochondrial function was inconstantly affected through acute bouts of exercise, most of the studies, which included training interventions, reported a higher mitochondrial function in immune cells, which were in some studies correlated with the CRF. In various disease conditions, mitochondrial function of immune cells was impaired compared to healthy controls.
- **Discussion:** If a regular exercise training is followed by an improvement in immune cell mitochondrial respiration, regular exercise could become even more important in the context of optimizing immune function and immune regulation in prevention and therapy.

KEY WORDS:
Physical Activity, Mitochondrial Respiration, Immune Cell Metabolism, Maximal Oxygen Uptake

Introduction

Cardiorespiratory fitness (CRF) is defined as the ability of the body's system to supply oxygen during sustained physical activity (19). CRF levels are strongly engulfed with health and disease and especially low levels of CRF are responsible for the highest percentage of all attributable fractions for all-cause mortality. In disease, both severity and prognosis are associated with patients' CRF levels (16).

It is well known that CRF can be modified by exercise training. Regular physical activity, preferably a structured training intervention can improve the CRF, mostly measured as the peak oxygen uptake in ml/kg/min ($\dot{V}O_2$peak). $\dot{V}O_2$peak displays a functional state of the whole organism and its central determinant is the cardiac output including heart rate and stroke volume. Peripheral determinants containing the arteriovenous oxygen difference including oxygen levels in vein and artery influenced i.e. by hemoglobin levels and the ability to metabolize oxygen efficiently. Consequently, improvement in $\dot{V}O_2$peak can result from a variety of adaptations commonly seen in the cardiovascular, hematologic, and metabolic systems (22).

When talking about CRF and $\dot{V}O_2$peak, mitochondria play a key role in oxygen-dependent metabolism. Mitochondria are present in all human cells except erythrocytes and represent the compartments in cells, where the metabolic pathway of oxidative phosphorylation (OXPHOS) and the electron transport chain (ETC) are located. In response to regular training an increased number and size of mitochondria with a functional adaptation of substrates and enzymes can be observed in various tissues and thus, affect the level of CRF (6, 27).

Even for immune cells, mitochondrial function is crucial for an efficient metabolism regarding host defense and tissue repair (8). Moreover, mitochondria are involved in the differentiation and activation processes of immune cells. Consisting of two sections, cells of the immune system can be divided into cells of the innate and adaptive immune system. Cells of the innate immune system include monocytes, macrophages/dendritic cells, granulocytes, as well as innate lymphocytic cells (ILCs) such as natural killer (NK) cells, and serve as the first line of defense against infections. T and B cells are ›

Article incorporates the Creative Commons Attribution – Non Commercial License.
https://creativecommons.org/licenses/by-nc-sa/4.0/

Scan QR Code and read article online.

CORRESPONDING ADDRESS:
Prof. Dr. Karsten Krüger
Justus Liebig University Giessen,
Department of Exercise Physiology and Sports Therapy, Institute of Sports Science
Kugelberg 62, 35394 Giessen, Germany
✉: Karsten.krueger@sport.uni-giessen.de

part of the adaptive immune response. While B cells can differentiate into antibody-producing plasma cells, T cells can be distinguished in immune response regulating and coordinating CD4+ T helper cells, and CD8+ cytotoxic T cells (1,8).

Immune cells show a high metabolic plasticity and also react metabolically very strongly to activating signals, such as those released during pathogen contact. As a result, they also react to various external stress factors, such as physical activity or exercise (23). After activation, immune cells have an increased energy demand and follow highly differentiated strategies to generate fuel. While activated monocytes, T- and B- cells mostly use glycolysis for Adenosine triphosphate (ATP) production, regulatory T cells or M2 macrophages more intensively use OXPHOS and beta-oxidation. Moreover, metabolic pathways of immune cells were found to be influenced by oxygen levels, inflammatory signaling molecules, and the availability of substrates. Distinct modifications in metabolic pathways are linked to immune effector functions, particularly in the production of specific sets of cytokines (25). It has been established that major immune cell functions, such as differentiation, cytokine production, proliferation, and cytotoxicity, are dependent on the metabolic function of each cell. However, depending on the cell type, subpopulation and activation state of the cell, the preferred energy source may vary (8).

Acute exercise can promote a slight activation of immune cells and thus elicit a shift of metabolic pathways (7). Here, exercise works in two directions: First, after a combination of prolonged and intensive exercise, a temporary decline of various immune cell functions was found, while second, a moderate training program over weeks improves immune functions in general (13, 15, 31). So far, the role of immune cell metabolism as a potential mediator and mechanism of modulation between exercise and altered function is poorly understood. In response to acute exercise, the systemic immune-inflammation index, which represents a score of platelets, neutrophils, and lymphocytes, increases, which indicates an exercise-induced pro-inflammatory response. At the same time, several inflammatory cytokines are released, which tend to initiate pro-inflammatory processes followed by an anti-inflammatory counter-regulation (36).

Several hypotheses are conceivable to answer this question: First, the improvement in $\dot{V}O_2$peak is linked to the mitochondrial mass not only in muscles but also in immune cells, which increases during exercise and increases overall cellular respiration. Second, each session provides a slight inflammatory and thereby metabolic stimulus, leading to an adaptive process in the form of an improved long-term performance of the cells. If immune cell mitochondrial metabolism increases with CRF, this might indicate that functional adaptations are also reflected at the level of immune cells and their subcellular structures, which could have major relevance to health. A link between mitochondrial dysfunctions in immune cells and the induction of several diseases, such as rheumatoid arthritis or diabetes is assumed (8). Within this context, the relevance of exercise is particularly highlighted, considering that physical activity is probably the most effective intervention to improve mitochondrial function and therefore represents a major approach for long-term health and therapies (27).

Today, the function of cells can be measured at a cellular level using various methods. The Oxygraph (Oroboros-2k, Innsbruck, Austria) for high-resolution respirometry and the extracellular flux analysis (Seahorse XF96 analyzer, Agilent, Santa Clara, CA) are pioneering in this regard. By experimental manipulation, single metabolic pathways can be influenced and oxygen consumption and metabolic flow can be recorded (29). Another novel method for measuring global metabolic function in immune cells by use of flow cytometry is called SCENITH (Single Cell EnergetIc metabolism by profilIng Translation inHibition), which has recently been validated. It is for profiling cellular metabolic responses at the single cell level that allows simultaneous analysis of multiple cell types using flow cytometry. This is mentioned here for completeness only but has not yet been applied in association with CRF (3).

In this narrative review, we aim to provide an overview of the studies that have investigated mitochondrial respiration in different immune cell populations in association with CRF. In a next step, we will discuss the effects of acute exercise as well as chronic exercise on immune cells' respiration. Particularly the distinction between the mitochondrial responses in acute vs. chronic effects, effects of CRF, and healthy vs. diseased will be considered. Taken together, we want to examine the current state of the role of cardiopulmonary fitness in immune cell metabolism in human adults.

Methods

This narrative review was guided by the research question, "What is known about the effects of acute exercise, chronic exercise, and physical fitness on mitochondrial immune cell metabolism in human adults?" To answer this question, the following inclusion criteria were made: (1) measurement of mitochondrial respiration in immune cells; (2) a documented fitness level ($\dot{V}O_2$peak) or disease severity assessment; (3) immune cells included were peripheral blood mononuclear cells (PBMCs), which consisting of monocytes, dendritic cells, and lymphocytes (T-, B-, and NK cells), lymphocytes, and isolated T cells, B cells, NK cells, platelets, and neutrophils; (4) bioenergetics of immune cells must have been measured with either Oxygraph or Seahorse; (5) We also decided to include intervention studies using physical activity, endurance exercise protocols, or other studies, which measuring performance outcomes, but no explicit resistance training studies. The database PubMed was used for study collection. Studies were excluded if (1) the inclusion criteria were not met, (2) did not report original data, or (3) did not include human participants.

Results

In total, we used data from 20 studies of which 12 studies included PBMCs, 2 studies with lymphocytes, 2 studies with T cells, one study with NK cells, two studies with platelets, and one study with neutrophils. No studies include whole leukocytes or only B cells. In the following section, results of studies will be classified in acute vs. chronic effects of exercise on immune cell metabolism, effects of CRF, and finally a focus on healthy vs. diseased subjects. Table 1 gives an overview of all studies and their interventions, while table 2 displays the results of these studies (see Table 1 and Table 2, and also suplementary material online).

PBMCs

In total, we found 12 studies, which analyzed mitochondrial metabolism in PBMCs. Three studies included an intervention approach, of which one intervention used HIIT training (10). The remaining studies used a reduced sitting intervention (24) or an aerobic endurance training program (14). Four studies examined the effects of a single bout of exercise, of which one study used a maximal exercise test (32), and the remaining

Table 1

Study interventions. IG=Intervention group; HC=Healthy controls; CG=Control group; HIIT=High-Intensity-Interval-Training; MICT=Moderate-intensity training; PAH=Pulmonary artery hypertension.

AUTHOR, YEAR	CELL TYPE	STUDY POPULATION	PARTICIPANTS (N)	$\dot{V}O_2$PEAK AT BASELINE (ML/KG/MIN)	EXERCISE PROGRAM
Janssen et al. (2022)	PBMCs	Healthy; young females adults	N: 31 (high fit: 15, low fit: 16)	High fit: 50.4 (49.0 – 54.0) Low fit: 35.1 (32.2 - 35.7)	Single bout of exercise
Liepinsh et al. (2020)	PBMCs	Healthy sedentary adults	N: 12	Mean: 33.3 ± 1.3	Low-intensity cycling exercise
Stampley et al. (2023)	PBMCs and subtypes	Collegiate swimmers	N: 11	-	Maximal exercise swimming bout
Theall et al. (2021)	PBMCs	Healthy active adults	N: 21	Mean: 36.5 ± 6.3	Single bout of exercise
Hedges et al. (2019)	PBMCs	Young healthy men	N: 10	-	HIIT: 3 days/week, 2 weeks
Kocher et al. (2017)	PBMCs	Sedentary HIV+ patients	N: 7	-	Light Aerobic exercise: 3 days/week, 12 weeks
Noz et al. (2019)	PBMCs	Participants with increased cardiovascular risk	N: 16	-	Low-intensity physical activity, 16 weeks
Brand et al. (2020)	Lymphocytes	Occupational burnout	N: 24 (IG: 12, HC: 12)	-	Intervention involving physical acitivity: 3 days/week, 12 weeks
Tsai et al. (2016)	Lymphocytes	Healthy sedentary males	N: 60 (HIIT: 20, MICT: 20, CG: 20)	HIIT: Mean: 34.0 ± 1.4 MICT: Mean: 33.1 ± 1.2 CG: Mean: 32.2 ± 1.0	HIIT or MICT under hypoxic stress: 5 days/week, 6 weeks
Andonian et al. (2022)	CD4+ T cells	Rheumatoid arthritis	N: 12	Mean: 24.9 ± 6.6	HIIT: 3 days/week, 10 weeks
Lin et al. (2022)	NK cells	Sedentary young males	N: 60 (HIIT: 20, MICT: 20, CG: 20)	HIIT: Mean: 33.5 ± 4.8 MICT: Mean: 33.2 ± 4.5 CG: Mean: 32.2 ± 4.1	HIIT or MICT: 5 days/week, 6 weeks
Hsu et al. (2019)	Platelets	Stroke patients	N: 30 (IG: 15, CG: 15)	mL/min: IG: Mean: 11.55 ± 8.0 CG: Mean: 11.65 ± 7.6	Cycling + general rehabilitation: 5 days/week, 4 weeks
Bartlett et al. (2020)	Neutrophils	Prediabetic overweight-obese older adults	N: 16 (IG: 10, HC: 6)	-	Low-volume high-intensity interval walking: 3 days/week, 10 weeks
Li et al. (2015)	PBMCs	Early-stage heart failure patients	N: 49 (IG: 25, HC: 24)	-	-
Shirakawa et al. (2019)	PBMCs	Mild CHF (NYHA class I-II) or moderate to severe CHF (NYHA class III)	N: 62 NYHA class I-II: 31 NYHA class III: 31	NYHA class I-II: Mean: 19.5 ± 4.6 NYHA class III: Mean: 14.5 ± 4.3	-
Sommer et al. (2022)	PBMCs	Pulmonary Arterial Hypertension	N: 39 In patient PAH: 14 Outpatient PAH: 15 CG: 10	-	-
Tyrrell et al. (2015)	PBMCs	Overweight, obese older adults	N: 15	-	-
Zhou et al. (2020)	PBMCs	Heart failure, hospitalized patients	N: 38 (IG: 19, HC: 19)	-	-
Gamradt et al. (2021)	T cells	Major depressive disorder	N: 56 (IG: 28, HC: 28)	-	-

three studies used exercise programs with lower intensities (12, 18, 33). All other studies presented cross-sectional data (17, 28, 30, 35, 37).

Acute vs. Chronic Effects of Exercise

A single bout of exercise (70% $\dot{V}O_2$peak, 60 minutes) did not significantly affect mitochondrial respiration in young females (12) while Liepinsh et al. showed increased basal respiration, and fatty-acid- dependent leak and OXPHOS respiration after one hour of low-intensity exercise (50W, 60 minutes) in sedentary adults (18). Other data showed that an acute bout of exercise (65-75% $\dot{V}O_2$max, 30 minutes) induced no changes in mitochondrial respiration in healthy active adults, but tissue-oxygen flow (oxygen flow multiplied with concentration in 1 ml of blood) showed increased basal, leak, OXPHOS, and maximal respiration after exercise (33). In swimmers, a maximal exercise test was followed by elevated basal cellular respiration, but no effects were ›

Table 2

Overview and summary of the findings across the included studies. OXPHOS=Oxidative phosphorylation; C=Correlation.

AUTHOR, YEAR	CELL TYPE	VARIABLES	ACUTE	TRAINING	IG VS. CG	CORRELATION
Janssen et al. (2022)	PBMCs	Basal respiration	↔	-	↑	-
		Maximal respiration	↔	-	↑	-
Liepinsh et al. (2020)	PBMCs	Basal respiration	↑	-	-	-
		OXPHOS	↑	-	-	-
Stampley et al. (2023)	PBMCs	Basal respiration	↑	-	-	-
Theall et al. (2021)	PBMCs	Total mitochondrial respiration	↔	-	-	-
Hedges et al. (2019)	PBMCs	$\dot{V}O_2$peak	-	↑	-	-
		Total mitochondrial respiration	-	↔	-	-
Kocher et al. (2017)	PBMCs	$\dot{V}O_2$peak	-	↑	-	No C. between $\dot{V}O_2$peak and mitochondrial function.
		Basal respiration	-	↔	-	
		Total mitochondrial respiration	-	↑	-	
Noz et al. (2019)	PBMCs	Basal respiration	-	↔	-	-
		Maximal respiration	-	↓	-	
Brand et al. (2020)	Lymphocytes	Total mitochondrial respiration	-	↑	↓	Positive C. between symptoms of depression and burnout with mitochondrial activity.
		Maximal respiration	-	↑	↓	
Tsai et al. (2016)	Lymphocytes	$\dot{V}O_2$peak	-	↑	↑	-
		Maximal respiration	-	↑	↑	
Andonian et al. (2022)	CD4+ T cells	$\dot{V}O_2$peak	-	↑	-	Positive C. between $\dot{V}O_2$peak and changes in basal and maximal respiration.
		Total mitochondrial respiration	-	↔	-	
Lin et al. (2022)	NK cells	$\dot{V}O_2$peak	-	-	-	Positive C. between changes of $\dot{V}O_2$peak and changes in reserve and maximal respiration.
		Maximal respiration	↑	↑	-	
Hsu et al. (2019)	Platelets	$\dot{V}O_2$peak	-	↑	↑	Positive C. between changes of $\dot{V}O_2$peak and changes in OXPHOS and maximal respiration.
		Maximal respiration	-	↑	↑	
Lin et al. (2021)	Platelets	$\dot{V}O_2$peak	-	↑	↑	Positive C. between changes of $\dot{V}O_2$peak and changes of OXPHOS and maximal respiration.
		Maximal respiration	-	↑	↑	
Bartlett et al. (2020)	Neutrophils	Basal respiration	-	↑	-	Negative C. between relative $\dot{V}O_2$peak and changes in HbA1c and fasting glucose.
		Maximal respiration	-	↑	-	Positive C. between relative $\dot{V}O_2$peak and fasting insulin.
Li et al. (2015)	PBMCs	Total mitochondrial respiration	-	-	↓	Negative C. between mitochondrial respiratory function and oxidative stress and inflammation.
		Basal respiration	-	-	↓	Negative C. between mitochondrial respiratory function and TNF-α, IL-6, CRP, SOD.
		Maximal respiration	-	-	↓	Negative C. between mitochondrial respiratory function and cardiometabolic risk factors.
Shirakawa et al. (2019)	PBMCs	$\dot{V}O_2$peak	-	-	↓	Negative C. between $\dot{V}O_2$peak and mitochondrial ROS levels.
		Maximal respiration	-	-	↓	
Sommer et al. (2022)	PBMCs	Mitochondrial respiration	-	-	↔	Negative C. between mitochondrial respiration and disease severity.
Tyrell et al. (2015)	PBMCs	-	-	-	-	Positive C. between spare respiratory capacity and maximal respiration and knee extensor strength.
Zhou et al. (2020)	PBMCs	Maximal respiration	-	-	↓	Negative C. between basal respiration, maximal respiration, spare respiratory capacity and IL-6.
Gamradt et al. (2021)	T cells	Total mitochondrial respiration	-	-	↓	-
		Basal respiration	-	-	↓	Negative C. between basal respiration, ATP-linked respiration, spare respiratory capacity and clinical-rated depression severity.

found for leak, OXPHOS, and maximal respiration. Here, tissue-oxygen flow exhibited elevated respiration states except leak respiration (32).

Effects of CRF

Janssen et al. investigated differences in PBMC (peripheral blood mononuclear cells) mitochondrial metabolism between high-fit ($\dot{V}O_2$peak ≥47) and low-fit ($\dot{V}O_2$peak ≤37) young female athletes (12). They found a higher basal and maximal respiration capacity, as well as a higher spare respiratory capacity, ATP-linked respiration, and proton leak in high-fit females. PBMC composition was shown to be similar between the high-fit and low-fit athletes (12). Hedges et al. found an increased $\dot{V}O_2$peak after two weeks of HIIT in young untrained men, but without any effects on body composition, weight or PBMC composition. Here, no changes in the mitochondrial respiration of PBMCs were found (10). In heart failure patients, cross-sectional data showed that mitochondrial ROS levels were inversely correlated with the $\dot{V}O_2$peak (28). While $\dot{V}O_2$peak and mitochondrial function of PBMCs increased significantly after twelve weeks of aerobic training in patients with HIV+, no correlation was found between both (14).

Healthy vs. Diseased

Three studies investigated the mitochondrial function in heart failure patients (17, 28, 37). Compared to healthy controls, mitochondrial respiration was impaired in patients with heart failure (17, 37). In addition, correlations between mitochondrial respiratory function and oxidative stress, inflammation, and cardiometabolic risk factors were found (17). Especially in one study, TNF-α, IL-6, CRP were inversely correlated with mitochondrial respiration (17). Another study in patients with heart failure showed that systemic inflammation is causally related to the mitochondrial function of PBMCs. An increase in NAD levels was able to improve mitochondrial respiration and attenuate the proinflammatory activation of PBMCs (37). In a study from Shirakawa et al., patients with moderate to severe heart failure (NYHA III group) showed lower mitochondrial respiratory capacity, including maximal respiration, compared to patients with mild heart failure (NYHA I-II) (28). Sommer et al. revealed no different mitochondrial respiration in pulmonary hypertension patients compared to their control group, but they found that the mitochondrial respiration in PBMCs of those patients was inversely correlated with disease severity (30). A positive correlation between basal respiration and knee extensor maximal strength was reported in overweight, obese older adults, while basal, maximal, and spare respiratory capacity wasinversely correlated with plasma IL-6 levels (35). In patients with HIV+, twelve weeks of aerobic training were followed by an increase in mitochondrial function, especially in respiration capacity, spare respiratory capacity, and non-mitochondrial respiration. A 16-week reduced sitting intervention in patients with increased cardiovascular risk was followed by a decrease in maximal respiration in PBMCs (24).

Lymphocytes

Two studies included the whole lymphocyte population in its analysis. Tsai et al. compared high- intensity interval training (HIIT) to moderate-intensity training (MICT), both under hypoxic conditions (34). Brand et al. examined the influence of regular physical activity on mitochondrial function in patients with occupational burnout (5).

Acute vs. Chronic Effects of Exercise and Effects of CRF in Health and Disease

The study of Tsai et al. investigated the acute and long-term effects of HIIT (n=20) and MICT (n=20) under hypoxic stress in healthy sedentary males and in a control group (n=20). Here, an acute bout of exercise at 12% O2 significantly decreased the ATP-linked respiration and reserve capacity. After six weeks of HIIT or MICT, a significantly higher $\dot{V}O_2$peak, but no change in mitochondrial count was found. Therefore, mitochondrial respiration was significantly improved by enhanced OXPHOS in both groups, but no correlation between $\dot{V}O_2$peak and mitochondrial respiration was detected (34).

In the study of Brand et al. 24 males between 36 and 65 years with occupational burnout (n=12) and a healthy control group (n=12) were recruited. The intervention group completed a 12-week training intervention with three 60-minute exercise bouts per week (60-75% of HF_{max}). An increased maximal respiration capacity, improved ATP levels and an upregulation of mitochondrial activity in lymphocytes after the intervention time were found. Patients with burnout had lower ATP levels and dysfunctional mitochondrial activity compared to a healthy control group. Moreover, higher baseline levels of mitochondrial activity were correlated with lower depression and burnout scales at baseline and after a 12-week physical activity intervention. Although the intervention led to improved mitochondrial activity and reduced symptoms of depression, these levels were still higher than those of the control group (5).

T Cells

Two studies analyzed mitochondrial respiration in T cells. In the study of Andonian et al., a 10-week HIIT intervention was conducted in patients with rheumatoid arthritis (n=12) (2), while the study of Gamradt et al. examined T cells of patients with major depressive disorder (MDD) (n=28) in a cross sectional design by using a control group (n=28) (9).

Acute vs. Chronic Effects of Exercise and Effects of CRF in Health and Disease

A 10-week HIIT intervention in patients with rheumatoid arthritis was followed by no improvements in mitochondrial respiration in CD4+ T cells, while improvements of mitochondrial respiration in CD4+ T cells were positively associated with naïve CD4+ T cell count. In addition, positive correlations between the CRF ($\dot{V}O_2$peak) and the basal and maximal mitochondrial respiration of CD4+ T cells were found. The mitochondrial respiration of CD4+ T cells in patients with rheumatoid arthritis seemed to be impaired whereas a HIIT intervention improved mitochondrial respiration in those cells (2).

The other study determined a reduced respiratory capacity in patients with non-overweight MDD. Moreover, the patients showed no differences in global shifts in T cell phenotypes or immune senescence (measured by flow cytometry) compared to the control group. The clinical-rated depression severity was inversely correlated with T cells' basal respiration, ATP linked respiration, and spare respiratory capacity (9).

NK Cells

One study about the effects of exercise and the metabolism of NK cells was found. Lin et al. carried out a six week HIIT (n=20) or MICT (n=20) in sedentary young males while using a graded exercise test before and after the interventions. Also, a control group (N=20) was included (21). ›

Acute vs. Chronic Effects of Exercise and Effects of CRF in Health and Disease

After the acute graded exercise test, the reserve and maximal respiration capacity of NK cells increased. Six weeks of HIIT or MICT were followed by both an enhanced reserve and maximal respiration capacity in NK cells of the resting participants. In addition, the authors showed a positive correlation between the change in $\dot{V}O_2$peak and the changes in reserve and maximal respiration capacity following HIIT and MICT (21).

Other Cell Types

Two studies investigated the bioenergetics of platelets. Hsu et al. investigated an aerobic 4-week exercise training program in stroke patients (n=15) in comparison to a control group (n=15) (11). The other study performed a 12-week aerobic rehabilitation intervention in patients with peripheral artery disease (n=20) in comparison with a control group (n=20) (20).

For neutrophils, one study by Bartlett et al. used a 10 week low-volume HIIT to determine mitochondrial function in prediabetic overweight-obese older adults (n=10) compared to a control group consisting of healthy young adults (n=6) (4).

Acute vs. Chronic Effects of Exercise and Effects of CRF in Health and Disease

No effects of acute exercise on mitochondrial respiration were described for either platelets or neutrophils.

In stroke patients, four weeks of exercise training lead to an increase of OXPHOS and maximal respiration of platelets compared to a control group. Moreover, positive correlations in the changes in $\dot{V}O_2$peak and the changes in OXPHOS and maximal respiration in platelets were found (11).

Even in patients with peripheral artery disease, twelve weeks of aerobic rehabilitation training in patients led to an increase of OXPHOS and maximal respiration of platelets. Furthermore, Lin et al. revealed an elevated $\dot{V}O_2$peak after 12 weeks of training and positive correlations with changes in the $\dot{V}O_2$peak and OXPHOS and maximal respiration of platelets in comparison to a control group (20).

For neutrophils, elevated basal respiration, maximal respiration, and a higher ATP production was found following 10 weeks of low-volume HIIT in prediabetic obese older adults. Here, young healthy adults showed an overall higher mitochondrial function (including basal and maximal respiration and ATP production) compared to the prediabetic intervention group. However, ten weeks of HIIT were followed by an increase in overall mitochondrial function and ensured that the values approximated those of the healthy young control group (4).

Discussion

This review article intends to investigate a potential connection between acute exercise, cardiorespiratory fitness and the metabolic capacity of different immune cell populations. In the field of exercise immunology, this question is highly relevant, as mitochondrial immune cell metabolism is suggested to represent a potential mechanism of modified immune function through acute or chronic exercise. Such a connection is not improbable, since especially endurance exercise is known to be a metabolic challenge for the entire organism, which also affects the availability of metabolites in blood and tissues, including cells of the immune system. Regular endurance training is also well known to induce numerous signals of increased mitochondrial biogenesis in tissues, which in turn influence CRF.

Four studies examined the effect of acute exercise bouts, which revealed inconsistent results in their effects on mitochondrial respiration. While two studies found no changes in mitochondrial respiration in PBMCs, the other two studies found higher routine respiration after an acute exercise bout. Regarding the exercise protocol, Theall et al. only used a 30 minutes exercise at an intensity of 65 to 75% of $\dot{V}O_2$peak (33). Another study obtained similar results in CD4+ T cells by a 30 minutes exercise intervention, and the authors argued whether the exposure was too low for acute alterations in mitochondrial respiration (26). However, time point of blood sampling can underlie these varying results. Overall, there is no clear evidence for an effect of acute exercise on metabolic performance immune cells, which might be explained by a too weak metabolic and inflammatory stimulus of the protocols used (26). We assumed that acute exercise influences cellular respiration through the exercise-induced immune response, primarily based on the release of (pro-)inflammatory cytokines. However, this does not seem to be consistently the case. Apparently, the inflammatory stimulus is not strong enough to sufficiently address the metabolic pathways in the immune cells. On the one hand, not enough signaling molecules are released by acute exercise to significantly influence cellular respiration. Also, the selected acute exercise protocols probably do not change the substrate availability to such an extent that the cells are in a significantly altered metabolic environment. On the other hand, the measurement methods used may not be sensitive enough to easily detect metabolic shifts.

In contrast, a majority of studies demonstrate that regular training affects immune metabolism at different levels, depending on the duration of the interventions. Most studies in which aerobic training was used as an intervention and the training duration exceeded two weeks, showed an increase in mitochondrial respiration for different immune cell types in parallel to an increase in $\dot{V}O_2$peak. Some studies controlled a potential shift of immune cell subpopulations as a cause for the altered respiration. However, in this cases, a similar cell composition compared to the control groups was found (9,10,12). Besides the potential shift of subpopulations, there might be molecular adaptations at the cellular level, resulting in an enhanced metabolic performance at the individual cell level. In this context, adaptations in molecular signaling pathways or subcellular structures, such as mitochondria, are discussed.

Since many diseases are also associated with a change in $\dot{V}O_2$peak, studies, which included different patient collectives, were also included in this article. Various diseases seem to be associated with a reduced immune cell respiration. Such diseases were often linked with a low-grade inflammation and mitochondrial dysfunction, which might represent important modulators of cellular respiration. For example, patients with rheumatoid arthritis showed defective mitochondrial DNA repair in T cells, which is associated with lower mitochondrial respiration (8). In patients with heart failure, early stages still seem to have a more functional immune metabolism, whereas this deteriorates in later stages. Other studies suggest associations between disease severity, risk factors, and mitochondrial respiration. An improvement of CRF has already been linked to enhanced effector function of immune cells. At this point, the discussion always comes up whether the immune cells adapt or simply operate more efficiently in an altered tissue environment. However, an improved immune cell metabolism, i.e. through a better nutrient availability is a potential mechanism here. Changes in the regulation of the mechanistic target of rapamycin (mTOR) or AMP kinase activity represent potential

mechanisms for an altered respiration. While mTOR is considered as a central metabolic regulator of immunity, AMP kinase promotes catabolism and inhibits mTOR, and limits immune cell activation (25). Even growth factors, cytokines, and pathogen-associated molecular patterns (PAMPs) can regulate the metabolic adaptations, growth, proliferation, and function of immune cells (8).

Overall, it appears that improved CRF may be associated with changes in immune cell metabolism. Of course, this must be further investigated with well-structured and controlled studies, as the existing studies still have many weaknesses. Different cell populations were examined, which at the same time still unite metabolically very heterogeneous subpopulations. As mentioned in the methods section, mostly studies on PBMCs were considered, which include cell types with a heterogeneous metabolism. Furthermore, subjects included are very heterogeneous and, in many studies, not characterized with regard to their CRF. The type of exercise programs is also very inconsistent in terms of type, duration and intensity. It would certainly be important to pursue these questions further. As this review is a narrative review, we tried to give an overview about the current knowledge in the field of mitochondrial immunometabolism and exercise, but there is a lack of systematic research, which is strongly needed.

It is difficult to derive practical recommendations from the current study situation. The data provide an indication, that a regular and long-term endurance training three times per week and at least 30 minutes at 60-70% of $\dot{V}O_2$peak lead to an adaptive response the metabolic function of certain immune cell subpopulations, but further research is needed. Moreover, it seems important, to assess the CRF (i.e. as the $\dot{V}O_2$peak) in clinical and experimental studies. If the increase in CRF does causally influence the metabolic competence of immune cells, it raises the question of how this, in turn, is directly associated with improved immune cell function and clinical outcomes. This may include factors like susceptibility to infection, antibodies in response to vaccination, or systemic immune regulation. In this case, regular exercise could become even more important in the context of optimizing immune function in prevention and therapy. ■

Conflict of Interest

The authors have no conflict of interest.

Summary Box

Acute bouts of exercise inconstantly affect mitochondrial immune cell respiration, while training interventions over weeks lead to a higher mitochondrial function in immune cells, which were in some cases correlated with the CRF. In various disease conditions, mitochondrial function of immune cells was impaired compared to healthy controls.

These findings highlight the potential role of regular endurance training and cardiorespiratory fitness in optimizing immune function, both in preventive and therapeutic contexts.

References

(1) **ABBAS AK, Lichtman AH, Pillai S.** Cellular and molecular immunology international edition. 9th ed. ELSEVIER - HEALTH Science; 2017. ISBN: 9780323523240.

(2) **Andonian BJ, Koss A, Koves TR, Hauser ER, Hubal MJ, Pober DM, Lord JM, MacIver NJ, St Clair EW, Muoio DM, Kraus WE, Bartlett DB, Huffman KM.** Rheumatoid arthritis T cell and muscle oxidative metabolism associate with exercise-induced changes in cardiorespiratory fitness. Sci Rep. 2022; 12: 7450. doi:10.1038/s41598-022-11458-4

(3) **Argüello RJ, Combes AJ, Char R, Gigan JP, Baaziz AI, Bousiquot E, Camosseto V, Samad B, Tsui J, Yan P, Boissonneau S, Figarella-Branger D, Gatti E, Tabouret E, Krummel MF, Pierre P.** SCENITH: A Flow Cytometry-Based Method to Functionally Profile Energy Metabolism with Single-Cell Resolution. Cell Metab. 2020; 32: 1063-1075.e7. doi:10.1016/j.cmet.2020.11.007

(4) **Bartlett DB, Slentz CA, Willis LH, Hoselton A, Huebner JL, Kraus VB, Moss J, Muehlbauer MJ, Spielmann G, Muoio DM, Koves TR, Wu H, Huffman KM, Lord JM, Kraus WE.** Rejuvenation of Neutrophil Functions in Association With Reduced Diabetes Risk Following Ten Weeks of Low-Volume High Intensity Interval Walking in Older Adults With Prediabetes - A Pilot Study. Front Immunol. 2020; 11: 729. doi:10.3389/fimmu.2020.00729

(5) **Brand S, Ebner K, Mikoteit T, Lejri I, Gerber M, Beck J, Holsboer-Trachsler E, Eckert A.** Influence of Regular Physical Activity on Mitochondrial Activity and Symptoms of Burnout-An Interventional Pilot Study. J Clin Med. 2020; 9: 667. doi:10.3390/jcm9030667

(6) **Carter HN, Chen CCW, Hood DA.** Mitochondria, muscle health, and exercise with advancing age. Physiology (Bethesda). 2015; 30: 208-223. doi:10.1152/physiol.00039.2014

(7) **Contrepois K, Wu S, Moneghetti KJ, Hornburg D, Ahadi S, Tsai MS, Metwally AA, Wei E, Lee-McMullen B, Quijada JV, Chen S, Christle JW, Ellenberger M, Balliu B, Taylor S, Durrant MG, Knowles DA, Choudhry H, Ashland M, Bahmani A, Enslen B, Amsallem M, Kobayashi Y, Avina M, Perelman D, Schüssler-Fiorenza Rose SM, Zhou W, Ashley EA, Montgomery SB, Chaib H, Haddad F, Snyder MP.** Molecular Choreography of Acute Exercise. Cell. 2020; 181: 1112-1130.e16. doi:10.1016/j.cell.2020.04.043

(8) **Faas MM, de Vos P.** Mitochondrial function in immune cells in health and disease. Biochim Biophys Acta Mol Basis Dis. 2020; 1866: 165845. doi:10.1016/j.bbadis.2020.165845

(9) **Gamradt S, Hasselmann H, Taenzer A, Brasanac J, Stiglbauer V, Sattler A, Sajitz-Hermstein M, Kierszniowska S, Ramien C, Nowacki J, Mascarell-Maricic L, Wingenfeld K, Piber D, Ströhle A, Kotsch K, Paul F, Otte C, Gold SM.** Reduced mitochondrial respiration in T cells of patients with major depressive disorder. iScience. 2021; 24: 103312. doi:10.1016/j.isci.2021.103312

(10) **Hedges CP, Woodhead JST, Wang HW, Mitchell CJ, Cameron-Smith D, Hickey AJR, Merry TL.** Peripheral blood mononuclear cells do not reflect skeletal muscle mitochondrial function or adaptation to high-intensity interval training in healthy young men. J Appl Physiol (1985). 2019; 126: 454-461. doi:10.1152/japplphysiol.00777.2018

(11) **Hsu C-C, Tsai H-H, Fu T-C, Wang J-S.** Exercise Training Enhances Platelet Mitochondrial Bioenergetics in Stroke Patients: A Randomized Controlled Trial. J Clin Med. 2019; 8: 2186. doi:10.3390/jcm8122186

(12) **Janssen JJE, Lagerwaard B, Porbahaie M, Nieuwenhuizen AG, Savelkoul HFJ, van Neerven RJJ, Keijer J, de Boer VCJ.** Extracellular flux analyses reveal differences in mitochondrial PBMC metabolism between high-fit and low-fit females. Am J Physiol Endocrinol Metab. 2022; 322: E141-E153. doi:10.1152/ajpendo.00365.2021

(13) **Knechtle B, Nikolaidis PT.** Physiology and Pathophysiology in Ultra-Marathon Running. Front Physiol. 2018; 9: 634. doi:10.3389/fphys.2018.00634

(14) **Kocher M, McDermott M, Lindsey R, Shikuma CM, Gerschenson M, Chow DC, Kohorn LB, Hetzler RK, Kimura IF.** Short Communication: HIV Patient Systemic Mitochondrial Respiration Improves with Exercise. AIDS Res Hum Retroviruses. 2017; 33: 1035-1037. doi:10.1089/aid.2016.0287

(15) **Kurowski M, Seys S, Bonini M, Del Giacco S, Delgado L, Diamant Z, Kowalski ML, Moreira A, Rukhadze M, Couto M.** Physical exercise, immune response, and susceptibility to infections-current knowledge and growing research areas. Allergy. 2022; 77: 2653-2664. doi:10.1111/all.15328

(16) **Lavie CJ, Ozemek C, Carbone S, Katzmarzyk PT, Blair SN.** Sedentary Behavior, Exercise, and Cardiovascular Health. Circ Res. 2019; 124: 799-815. doi:10.1161/CIRCRESAHA.118.312669

(17) **Li P, Wang B, Sun F, Li Y, Li Q, Lang H, Zhao Z, Gao P, Zhao Y, Shang Q, Liu D, Zhu Z.** Mitochondrial respiratory dysfunctions of blood mononuclear cells link with cardiac disturbance in patients with early-stage heart failure. Sci Rep. 2015; 5: 10229. doi:10.1038/srep10229

(18) **Liepinsh E, Makarova E, Plakane L, Konrade I, Liepins K, Videja M, Sevostjanovs E, Grinberga S, Makrecka-Kuka M, Dambrova M.** Low-intensity exercise stimulates bioenergetics and increases fat oxidation in mitochondria of blood mononuclear cells from sedentary adults. Physiol Rep. 2020; 8: e14489. doi:10.14814/phy2.14489

(19) **Liguori G, ed.** ACSM's guidelines for exercise testing and prescription. Philadelphia: Wolters Kluwer Health; 2022: 513. ISBN: 9781975150181

(20) **Lin ML, Fu TC, Hsu CC, Huang SC, Lin YT, Wang JS.** Cycling Exercise Training Enhances Platelet Mitochondrial Bioenergetics in Patients with Peripheral Arterial Disease: A Randomized Controlled Trial. Thromb Haemost. 2021; 121: 900-912. doi:10.1055/s-0040-1722191

(21) **Lin M-L, Hsu C-C, Fu T-C, Lin Y-T, Huang Y-C, Wang J-S.** Exercise Training Improves Mitochondrial Bioenergetics of Natural Killer Cells. Med Sci Sports Exerc. 2022; 54: 751-760. doi:10.1249/MSS.0000000000002842

(22) **Montero D, Diaz-Cañestro C, Lundby C.** Endurance Training and V˙O2max: Role of Maximal Cardiac Output and Oxygen Extraction. Med Sci Sports Exerc. 2015; 47: 2024-2033. doi:10.1249/MSS.0000000000000640

(23) **Nieman DC, Wentz LM.** The compelling link between physical activity and the body's defense system. J Sport Health Sci. 2019; 8: 201-217. doi:10.1016/j.jshs.2018.09.009

(24) **Noz MP, Hartman YAW, Hopman MTE, Willems PHGM, Tack CJ, Joosten LAB, Netea MG, Thijssen DHJ, Riksen NP.** Sixteen-Week Physical Activity Intervention in Subjects With Increased Cardiometabolic Risk Shifts Innate Immune Function Towards a Less Proinflammatory State. J Am Heart Assoc. 2019; 8: e013764. doi:10.1161/JAHA.119.013764

(25) **O'Neill LAJ, Kishton RJ, Rathmell J.** A guide to immunometabolism for immunologists. Nat Rev Immunol. 2016; 16: 553-565. doi:10.1038/nri.2016.70

(26) **Palmowski J, Gebhardt K, Reichel T, Frech T, Ringseis R, Eder K, Renner-Sattler K, Krüger K.** The Impact of Exercise Serum on Selected Parameters of CD4+ T Cell Metabolism. Immuno. 2021; 1: 119-131. doi:10.3390/immuno1030008

(27) **San-Millán I.** The Key Role of Mitochondrial Function in Health and Disease. Antioxidants (Basel). 2023; 12: 782. doi:10.3390/antiox12040782

(28) **Shirakawa R, Yokota T, Nakajima T, Takada S, Yamane M, Furihata T, Maekawa S, Nambu H, Katayama T, Fukushima A, Saito A, Ishimori N, Dela F, Kinugawa S, Anzai T.** Mitochondrial reactive oxygen species generation in blood cells is associated with disease severity and exercise intolerance in heart failure patients. Sci Rep. 2019; 9: 14709. doi:10.1038/s41598-019-51298-3

(29) **Smolina N, Bruton J, Kostareva A, Sejersen T.** Assaying Mitochondrial Respiration as an Indicator of Cellular Metabolism and Fitness. Methods Mol Biol. 2017; 1601: 79-87. doi:10.1007/978-1-4939-6960-9_7

(30) **Sommer N, Theine FF, Pak O, Tello K, Richter M, Gall H, Wilhelm J, Savai R, Weissmann N, Seeger W, Ghofrani HA, Hecker M.** Mitochondrial Respiration in Peripheral Blood Mononuclear Cells Negatively Correlates with Disease Severity in Pulmonary Arterial Hypertension. J Clin Med. 2022; 11: 4132. doi:10.3390/jcm11144132

(31) **Souza D, Vale AF, Silva A, Araújo MAS, de Paula Júnior CA, de Lira CAB, Ramirez-Campillo R, Martins W, Gentil P.** Acute and Chronic Effects of Interval Training on the Immune System: A Systematic Review with Meta-Analysis. Biology (Basel). 2021; 10: 868. doi:10.3390/biology10090868

(32) **Stampley JE, Cho E, Wang H, Theall B, Johannsen NM, Spielmann G, Irving BA.** Impact of maximal exercise on immune cell mobilization and bioenergetics. Physiol Rep. 2023; 11: e15753. doi:10.14814/phy2.15753

(33) **Theall B, Stampley J, Cho E, Granger J, Johannsen NM, Irving BA, Spielmann G.** Impact of acute exercise on peripheral blood mononuclear cells nutrient sensing and mitochondrial oxidative capacity in healthy young adults. Physiol Rep. 2021; 9: e15147. doi:10.14814/phy2.15147

(34) **Tsai H-H, Chang S-C, Chou C-H, Weng T-P, Hsu C-C, Wang J-S.** Exercise Training Alleviates Hypoxia-induced Mitochondrial Dysfunction in the Lymphocytes of Sedentary Males. Sci Rep. 2016; 6: 35170. doi:10.1038/srep35170

(35) **Tyrrell DJ, Bharadwaj MS, van Horn CG, Marsh AP, Nicklas BJ, Molina AJA.** Blood-cell bioenergetics are associated with physical function and inflammation in overweight/obese older adults. Exp Gerontol. 2015; 70: 84-91. doi:10.1016/j.exger.2015.07.015

(36) **Walzik D, Joisten N, Zacher J, Zimmer P.** Transferring clinically established immune inflammation markers into exercise physiology: focus on neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio and systemic immune-inflammation index. Eur J Appl Physiol. 2021; 121: 1803-1814. doi:10.1007/s00421-021-04668-7

(37) **Zhou B, Wang DD, Qiu Y, Airhart S, Liu Y, Stempien-Otero A, O'Brien KD, Tian R.** Boosting NAD level suppresses inflammatory activation of PBMCs in heart failure. J Clin Invest. 2020; 130: 6054-6063. doi:10.1172/JCI138538

6 Discussion

By addressing how acute exercise, CRF, inflammation, and metabolic status collectively affect mitochondrial respiratory states and function of immune cells, this cumulative dissertation provides insights that across young and elderly cohorts, the mitochondrial respiration of immune cells represents an important indicator of cellular health and function, thereby reflecting the quality of the immune cell's metabolism. In exercise physiology, particularly exercise immunology, these methodological approaches offer novel means to investigate mitochondrial function and metabolism in immune cells, which are pivotal determinants of systemic health and cellular homeostasis. The main results are discussed below, with consideration of their relevance within the broader scientific framework.

The ability of mitochondria to adapt to physiological challenges is essential for cellular responses, while proper activity of the mitochondrial respiratory chain is critical for the normal function of tissues with high energy demands, including the brain, heart, and skeletal muscle. Even immune cells require substantial energy upon activation, which is accompanied by pronounced changes in metabolism. Failure of these adaptive responses is closely linked to the onset of conditions characterized by impaired mitochondrial function, including metabolic and age-associated diseases, as well as cancer [48]. Therefore, the assessment of mitochondrial respiration, particularly in distinct immune cell populations, provides both clinical and scientific research with the opportunity to investigate cell-specific energy metabolism and to draw conclusions about underlying metabolic pathways involved in cellular energy turnover. Although HRR with the Oroboros O2k Oxygraph requires technically demanding protocols, it provides a robust and sensitive assessment of mitochondrial respiration, thereby complementing transcriptomic, metabolomic, and immunophenotyping analyses with functional information. Within this dissertation, altered respiratory parameters allow sensitive detection of metabolic alterations, enabling the observation of meaningful effects even in healthy cohorts.

As mitochondrial adaptations to exercise are predominantly studied in skeletal muscle, direct associations with immune cells appear limited. Both tissues share conserved signaling pathways involved in mitochondrial regulation; however, because mitochondrial function is shaped in a tissue-specific manner, their physiological roles differ fundamentally. In skeletal muscle, mitochondrial respiration primarily reflects the ability to sustain ATP production for mechanical work and metabolic performance, whereas in immune cells, mitochondrial respiration is tightly linked to activation state, differentiation, and inflammatory signaling (see chapters 3.1 and 3.2) [94, 95]. Human studies demonstrate that short-term high-intensity training increases skeletal muscle mitochondrial respiration without corresponding changes in

PBMC respiration, and no significant correlations between muscle and PBMC bioenergetics have been observed [96–98]. These findings suggest that circulating immune cells cannot be considered a direct surrogate marker of skeletal muscle mitochondrial adaptation. Furthermore, the metabolic heterogeneity of PBMC populations may obscure subset-specific adaptation [97]. Accordingly, metabolic adaptations in circulating immune cells should not be expected to directly reflect tissue-specific mitochondrial responses, as each cell type serves distinct physiological functions and responds to exercise in a context-dependent manner [9]. However, the muscle-immune crosstalk during exercise and its consequences should not be underestimated [99].

To interpret the present findings of this dissertation, mitochondrial respiratory adaptations must be considered within a multi-level framework encompassing the biological processes and regulatory pathways that shape mitochondrial respiration. Here, there are three levels at which mitochondrial respiratory adaptations occur: (1) main mechanisms of mitochondrial adaptation, summarized as all biological processes that adjust mitochondrial function, (2) key regulatory components as central molecular players that regulate those mechanisms, and (3) modulators, which are extrinsic and intrinsic stimuli activating key regulatory components and initiate the main mitochondrial adaptation mechanisms [48]. In this dissertation, major modulators of mitochondrial respiration in immune cells are addressed, including acute exercise, CRF, metabolic risk factors, inflammation, chronic diseases, and aging. Nevertheless, it is mostly unknown how precisely mitochondrial respiration is targeted by these modulators and how molecular mechanisms shape immune cell bioenergetics. This directs the discussion toward the functional characteristics of the different modulators of mitochondrial respiration across physiological and pathological contexts.

Acute exercise represents a transient metabolic and inflammatory perturbation that challenges systemic homeostasis and temporarily reshapes the metabolic microenvironment of immune cells. It markedly alters circulating metabolites, substrate availability, and cytokine profiles [59] (see chapter 3.3.1). Novel findings extend this concept. They show that immune cell mobilization and redistribution alter the proteome of PBMCs, which is related to effector function and immune cell activation pathways within one hour after exercise. Higher intensity leads to greater changes in more proteins in PBMCs after exercise and especially a temporary reduction in proteins associated with mitochondrial processes [100]. Given these systemic shifts, measurable changes in mitochondrial respiratory states of immune cells would appear plausible. However, the present findings of this dissertation indicate that acute exercise induces only limited and heterogeneous alterations in mitochondrial respiration of immune cells. Despite the use of sensitive methodologies to quantify oxygen consumption (e.g.,

PreSens or extracellular flux analyses by Seahorse), mitochondrial respiration appears comparatively stable during acute exercise [16, 67]. This may partly reflect differences in exercise protocols and sampling time points, but it also raises the possibility that mitochondrial respiration in immune cells is a relatively robust parameter that is not readily shifted by short-lived metabolic or inflammatory fluctuations. Given its central importance for host defense, immune cell metabolism may be buffered against transient systemic perturbations, thereby preserving bioenergetic function despite acute metabolic stress. For instance, T cells maintain their metabolic state through tightly regulated sensitivity to tonic signals, requiring a sufficiently strong stimulus for full activation [101]. Similarly, increases in inflammatory and, in particular, immunoregulatory cytokines following acute exercise could be an attempt to prevent an uncontrolled inflammatory response [70]. Such regulatory stability could contribute to the heterogeneous or minimal changes observed following single exercise bouts.

Although acute exercise can activate cellular energy-sensing pathways such as AMPK, these signaling responses should be interpreted as transient metabolic adaptations rather than structural mitochondrial remodeling [8]. However, Tsai et al. observed an increased mitochondrial membrane potential in lymphocytes after moderate as well as high-intensity exercise [102]. Acute exercise may primarily induce rapid shifts in substrate utilization and intracellular signaling networks instead of immediate changes in mitochondrial respiration, mitochondrial density, or mitochondrial function [59]. These findings suggest that acute exercise can modify mitochondrial signaling properties in immune cells without necessarily being accompanied by measurable changes in mitochondrial respiration.

In contrast to this transient nature of acute exercise, CRF emerged as a more consistent modulator of mitochondrial respiration in immune cells. Higher CRF was particularly associated with enhanced maximal respiration, suggesting increased respiratory capacity. These findings support the concept that mitochondrial respiration in immune cells is not an acute response, but rather the result of repeated metabolic challenges over time. Moreover, this interpretation is strengthened by both the present findings and complementary transcriptomic and metabolomic signatures, demonstrating an upregulation of ETS-related proteins and metabolic signaling pathways in individuals with higher CRF. In addition, in a recent investigation, a higher CRF was associated with an increased expression of several proteins linked to enhanced metabolic and functional capacity of PBMCs [100]. In line with this interpretation, recent evidence demonstrates that mitochondrial adaptations in immune cells are positively related to CRF and energy expenditure. Individuals with higher CRF and regular aerobic activity exhibit greater mitochondrial mass in naïve $CD8^+$ T cells compared with less active

individuals, indicating that improvements in CRF may be accompanied not only by functional but also structural mitochondrial remodeling within immune cells [5, 103].

The observed association with maximal respiration is particularly relevant, as this parameter reflects the upper limit of ETS and thus the maximal bioenergetic capacity available under stress conditions, such as pathogen exposure [53]. A higher ETC is commonly linked to increased mitochondrial density and/or enhanced enzymatic capacity of the ETS, which may further explain the observed association with CRF. Another possible explanation may also involve chronically increased systemic oxygen availability in fitter individuals, potentially activating signaling pathways that extend beyond skeletal muscle and influence immune cell metabolism [104]. These results emphasize the complex interplay between acute metabolic perturbations and chronic bioenergetic adaptation and reinforce the concept that CRF represents a central modulator of long-term mitochondrial remodeling in immune cells. CRF appears to reflect a chronic metabolic phenotype characterized by repeated activation of energetic stress pathways and sustained systemic adaptation. From a mechanistic perspective, CRF may therefore be interpreted as an integrative marker of long-term metabolic exposure and systemic metabolic health. Recurrent activation and thus, mild disturbance of homeostasis induced by exercise-related metabolic shifts may progressively activate energy-sensing pathways, mitochondrial biogenesis programs, and redox-regulatory networks, which could shape mitochondrial respiration in immune cells.

In this context and as a next potential modulator, metabolic risk factors may be conceptualized as an early stage of mitochondrial and metabolic dysregulation, representing chronic mitochondrial stressors beyond purely inflammatory stimuli. The associations observed in this dissertation between glucose, HbA1c, and immune cell mitochondrial respiration suggest that mitochondrial bioenergetics are sensitive to subtle metabolic disturbances already at subclinical stages. These observations are consistent with study findings demonstrating associations between cardiometabolic risk factors and mitochondrial respiration in PBMCs from generally healthy adults. In this cohort, higher LDL cholesterol was inversely related to maximal respiration, spare respiratory capacity, and overall metabolic potential of PBMCs, while elevated systolic blood pressure showed similar negative relationships with mitochondrial respiratory function. Importantly, these associations were independent of age, adiposity, and other lipid parameters, suggesting that even moderate cardiometabolic burden may adversely affect immune cell bioenergetics [105]. Even in young children (ages 4 to 8), whole-genome microarray analysis of PBMCs revealed early changes between normal weight and overweight individuals, characterized by downregulation of genes related to OXPHOS, TCA cycle, as well as dysregulation of glucose and lipid metabolism pathways. This suggests an impaired

metabolic handling and a potential predisposition to later obesity-associated metabolic disorders such as type 2 diabetes [106]. However, metabolic risk factors may initially impose mitochondrial stress through substrate overload and redox imbalance, challenging mitochondrial homeostasis and thus, subsequently contributing to the development of chronic inflammatory responses [107]. These findings support the notion that mitochondrial alterations in immune cells may occur early in the cardiometabolic continuum, preceding overt metabolic disease.

It is well known that inflammation induces immunometabolic reprogramming, characterized by shifts between OXPHOS and glycolysis on immune cell activation state (see chapter 3.1). Inflammation represents both a modulator and a consequence of altered mitochondrial respiratory function. The associations observed between immune cell mitochondrial respiration and inflammatory markers indicate that higher mitochondrial capacity aligns with a more anti-inflammatory phenotype, whereas reduced respiration frequently accompanies elevated IL-6 and TNF-α [108]. Impaired mitochondrial respiration may either contribute to or result from inflammatory processes, highlighting its dual role as both a driver and a biomarker of immunometabolic dysfunction. Mechanistically, impaired ETC may increase mitochondrial ROS production and disturb NAD^+ homeostasis, thereby activating inflammatory signaling pathways, such as NF-κB [107]. Conversely, persistent inflammatory signaling can disrupt mitochondrial coupling efficiency and progressively impair bioenergetic performance. This bidirectional relationship positions mitochondrial dysfunction as a central node linking inflammatory burden to functional decline in immune cells [55, 109]. Such mechanisms are particularly relevant in chronic disease, as early mitochondrial alterations arising in the context of sustained inflammation may constitute a mechanistic bridge to later immune dysregulation and chronic low-grade inflammation [58].

Within this dissertation, chronic disease states are frequently characterized by impaired immune cell bioenergetics, supporting the concept that mitochondrial respiratory dysfunction accompanies sustained inflammatory and metabolic burden. The findings of this dissertation revealed that patients often exhibit reduced basal and maximal mitochondrial respiration in PBMCs compared with healthy individuals, which is commonly associated with elevated inflammation markers (e.g., CRP, IL-6, TNF-α) and greater disease severity. At a mechanistic level, mitochondrial dysfunction and chronic inflammatory signaling may impair ETS efficiency via increased mitochondrial ROS production and disturbed NAD^+ homeostasis. In parallel, persistent immunometabolic reprogramming may reduce mitochondrial respiratory capacity of immune cells. Similar alterations have been described in metabolic diseases such as type 2 diabetes mellitus [110]. This indicates that mitochondrial respiration is more strongly affected

by chronic inflammatory conditions, such as chronic low-grade inflammation or metabolic disease, than by transient perturbations such as acute exercise.

Another potential modulator of mitochondrial respiration is aging. Although aging is accompanied by shifts in immune cell composition, changes in cellular compositions may partly explain alterations in metabolic profiles, as distinct immune subsets exhibit different energetic demands (see chapter 3.3.2). However, the currently heterogeneous evidence suggests that chronological age alone is not a consistent determinant of mitochondrial respiratory changes, as the maintenance of naïve T cell pools and lower inflammatory burden appear more closely linked to preserved mitochondrial respiration and mitochondrial function than chronological age per se [111]. This notion is further supported by observations from healthy aging cohorts, in which immune cell redistribution and subclinical vascular alterations were more strongly associated with metabolic and inflammatory profiles than chronological age [112]. Importantly, mitochondrial dysfunction is considered a hallmark of aging, yet a gradual decline in mitochondrial respiration has been consistently demonstrated primarily in skeletal muscle, whereas findings in immune cells remain inconsistent [111, 113]. In this context, metabolic risk factors and sustained inflammatory signaling may progressively impair mitochondrial quality control, leading to functional decline in immune cells [114]. Conversely, healthy aging, characterized by preserved metabolic homeostasis and low inflammatory tone, may allow the maintenance of mitochondrial respiration [115]. Together, these observations indicate that the impact of aging on immune cell mitochondrial respiration is largely context-dependent and strongly influenced by metabolic and inflammatory status, including cumulative exposure to metabolic risk, chronic low-grade inflammation, and adaptive counter-regulatory mechanisms across the lifespan.

Regular PA represents one of the few modifiable interventions shown to improve mitochondrial respiratory function of immune cells, particularly in patients with chronic low-grade inflammatory diseases. Although longitudinal exercise-intervention studies directly assessing exercise-induced changes in immune cell respiration remain scarce, available evidence consistently indicates an increase in mitochondrial respiratory states following structured training interventions, in some cases paralleling improvements in VO_2max [116, 117]. Notably, improvements in mitochondrial respiration have been reported alongside enhanced CRF and functional immunological and inflammatory adaptations, suggesting coordinated systemic and metabolic remodeling in response to exercise training [11]. Consistent with this view, an 18-week endurance training intervention in men induced pronounced alterations in PBMC gene expression, revealing transcriptional signatures linked to immune regulation, cell cycle control, and cellular development, while baseline transcriptional profiles were associated with

subsequent improvements in VO_2max [118]. Nevertheless, the causal nature of the positive association between CRF and maximal mitochondrial respiration of different immune cells remains to be clarified. It is still unresolved whether immune cell mitochondrial respiration directly reflects whole-body VO_2max or whether both parameters are parallel consequences of systemic adaptation. Given the established prognostic relevance of CRF for all-cause mortality and cardiometabolic health, the association with immune cell respiratory capacity raises the possibility that mitochondrial function in immune cells represents a cellular correlate to fitness resilience and thus provides additional evidence for the systemic importance of CRF and habitual physical activity [74, 119].

These findings reinforce the concept that mitochondrial respiration is not fixed but responsive to sustained metabolic stimulation, underscoring the relevance of an active and healthy lifestyle for maintaining immune bioenergetic resilience. Such adaptations may also be interpreted within the framework of 'mitohormesis', whereby mild, repeated metabolic or mitochondrial perturbations trigger compensatory responses that enhance mitochondrial function and cellular resilience to stress. This low-level stress, thus, results in long-term adaptations, including increased metabolic function and improved health [114, 115, 120]. Beyond exercise alone, an active and healthy lifestyle, combined with favorable behavioral factors, such as balanced nutrition, adequate sleep, and stress regulation, may attenuate metabolic risk profiles and thereby reduce mitochondrial strain [90]. In patients with chronic inflammatory conditions, such adaptations may translate into reduced inflammatory burden, lower disease severity, and improved clinical outcomes. This may, in part, be associated with improvements in mitochondrial respiration, thereby positioning immune cell mitochondrial respiration as both a mechanistic target and a marker of therapeutic response.

Taken together, mitochondrial respiration in immune cells may provide added value as an integrative marker of immunometabolic health. Its sensitivity to metabolic and inflammatory perturbations, even in healthy cohorts, suggests potential utility in the early detection of systemic dysregulation and in monitoring disease-related bioenergetic alterations in the context of physical activity and health. Importantly, its modulation by a spectrum of physiological and pathological factors, including exercise, CRF, metabolic risk factors, inflammation, chronic disease, and aging, highlights the role as a dynamic interface reflecting cumulative internal and external influences on cellular energy metabolism. In this context, a healthy lifestyle, particularly regular physical activity, may contribute to the maintenance of mitochondrial respiratory states and function and support immunometabolic stability over time.

7 Potential Relevance and Future Perspectives

Given the currently limited and partly inconsistent evidence, the functional and clinical role of mitochondrial respiration in immune cells remains difficult to interpret conclusively. Therefore, this section aims to outline the potential future relevance and added value of assessing mitochondrial respiration in immune cells and should not be interpreted as providing definitive conclusions. The concepts discussed are primarily exploratory and hypothesis-generating and must be interpreted with appropriate caution. Any proposed relevance remains tentative and requires further substantiation through rigorously designed studies. Importantly, the current evidence is largely associative, and a lack of causal data necessitates well-designed longitudinal and interventional studies to establish causality.

Modulation of mitochondrial respiration has functional consequences for immune cell adaptability. Enhanced respiration, particularly an increased maximal ETC, may provide greater bioenergetic flexibility, enabling immune cells to respond more efficiently to intrinsic and extrinsic stressors. Conversely, reduced mitochondrial respiration may limit metabolic flexibility and predispose immune cells to unresolved inflammatory responses under chronic metabolic or inflammatory stress. In this context, mitochondrial respiratory function appears less as a simple marker of activation and more as an indicator of immunometabolic resilience, reflecting the ability of immune cells to maintain homeostasis.

However, the functional relevance of altered mitochondrial respiration in immune cells through different modulators cannot be interpreted in absolute terms. Whether changes represent adaptive resilience or maladaptive dysfunction depends on the surrounding metabolic and inflammatory milieu and not solely on the magnitude of mitochondrial respiration. Mitochondrial respiration of immune cells and its modulation may have clinical relevance because (1) it determines immune functionality and cellular metabolism can be investigated in greater detail, allowing deeper insights into immune cell energetics, (2) it may predict disease severity, (3) it is associated with chronic inflammatory and metabolic diseases, and (4) it creates new therapeutic targets and biomarker opportunities in health and disease, such as in immunotherapies. However, the clinical applicability as a biomarker remains unclear and requires further validation.

In sports medicine and exercise immunology, mitochondrial respiration in immune cells may be used (1) as a research biomarker for systemic stress burden and inflammation, and (2) as a window into immune health. It appears less suitable for acute performance monitoring but may reflect chronic inflammation, infection, or metabolic stress, which is also highly important by indirectly influencing the performance of athletes.

Despite the growing evidence linking mitochondrial respiration of immune cells to metabolic health, several important mechanistic and translational questions remain unresolved. Future research should clarify how systemic metabolic modulators regulate immune cell bioenergetics and whether mitochondrial respiration represents a mechanistically relevant driver of immunometabolic adaptation. Integration of HRR with immunophenotyping and multi-omics approaches will be essential to improve mechanistic resolution and biomarker suitability [121].

A critical limitation of current approaches is the frequent use of heterogeneous cell populations such as PBMCs. Distinct immune cell subpopulations exhibit context-dependent differences in mitochondrial respiration, which may obscure biologically relevant effects. Future studies should therefore focus on cell-type-specific analyses (e.g., isolated $CD4^+$ T cells) to improve interpretability. Single-cell approaches, such as SCENITH, allow simultaneous assessment of metabolic dependencies across multiple immune cell subsets [122].

From a clinical perspective, interventional studies in both healthy and patient populations are required to determine whether immune cell mitochondrial respiration predicts disease risk, therapeutic responsiveness, or vaccination outcomes. Balanced inclusion of both sexes is essential to improve generalizability and account for sex-specific responses. All studies should also assess whether modulating immune cell mitochondrial function improves immune competence and reduces inflammatory burden.

In exercise physiology and sports medicine, well-controlled training studies are needed to determine the trainability of immune cell mitochondrial respiration and its relationship to skeletal muscle adaptations. Current evidence suggests that immune cell bioenergetics does not directly mirror muscle mitochondrial adaptations, underscoring the need for tissue-specific interpretation [96, 97]. Elite athletes represent a valuable model to examine the physiological limits of immune cell mitochondrial respiration. It remains unclear whether extreme training loads or states of low energy availability (LEA) or relative energy deficiency in sports (REDs) impair mitochondrial respiratory function in immune cells and contribute to immune vulnerability. Longitudinal studies in athletic populations are therefore needed to determine whether reductions in immune cell mitochondrial respiration indicate maladaptive training load or reduced immune resilience.

Finally, immune cell mitochondrial respiration should be interpreted within a broader immunometabolic framework to determine if it represents a marker of systemic fitness and a modifiable mediator of long-term metabolic and inflammatory health [121]. At present, its role as a reliable biomarker or therapeutic target remains to be clearly established.

8 Conclusion

This cumulative dissertation demonstrates that mitochondrial respiration of different immune cells (PBMCs and $CD4^+$ T cells) represents a sensitive and integrative marker of immunometabolic adaptation across physiological and pathological contexts. Besides the measurement of mitochondrial respiratory states, this dissertation gives insights into various important modulators, enabling their potential to affect mitochondrial respiratory function at different mechanistic levels. Acute exercise induces only minor and heterogeneous immediate effects, whereas higher CRF is consistently associated with enhanced maximal respiratory capacity, indicating that immune cell bioenergetics reflect cumulative metabolic exposure more than transient perturbations. Metabolic risk factors, inflammation, and chronic disease states are linked to impaired basal and maximal mitochondrial respiration in immune cells and subtypes, supporting the concept that mitochondrial dysfunction accompanies sustained immunometabolic strain. In contrast, as seen in healthy aging cohorts, preserved or enhanced mitochondrial respiration aligns with favorable immune composition, including higher counts of naïve $CD4^+$ T cells, lower inflammatory burden, and metabolic resilience. These findings position immune cell mitochondrial respiration at the intersection of physical fitness, inflammatory status, aging, and chronic disease. Importantly, mitochondrial respiration appears to be modulated more strongly by long-term metabolic and inflammatory conditions than by chronological age or short-term stimuli (e.g., acute exercise) alone. Future studies should investigate the effects of long-term exercise interventions to examine whether exercise can enhance mitochondrial respiration in both healthy individuals and patients. Collectively, immune cell mitochondrial respiration emerges as both a functional readout of cellular health and a potential mediator linking lifestyle-related factors to systemic metabolic and inflammatory outcomes. Nevertheless, mitochondrial respiration represents only one dimension of the highly complex and multi-layered nature of mitochondrial biology, underscoring the need for integrative and holistic approaches; despite reflecting only a part of the broader immunometabolic landscape, immune cell mitochondrial respiration has potential relevance as a functional biomarker for immune health.

References

1. Paludan SR, Pradeu T, Masters SL, Mogensen TH. Constitutive immune mechanisms: mediators of host defence and immune regulation. Nat Rev Immunol. 2021;21:137–50. doi:10.1038/ s41577-020-0391-5.
2. O'Neill LAJ, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. Nat Rev Immunol. 2016;16:553–65. doi:10.1038/nri.2016.70.
3. Hu C, Xuan Y, Zhang X, Liu Y, Yang S, Yang K. Immune cell metabolism and metabolic reprogramming. Mol Biol Rep. 2022;49:9783–95. doi:10.1007/s11033-022-07474-2.
4. Weinberg SE, Sena LA, Chandel NS. Mitochondria in the regulation of innate and adaptive immunity. Immunity. 2015;42:406–17. doi:10.1016/j.immuni.2015.02.002.
5. Alley JR, Valentine RJ, Kohut ML. Mitochondrial Mass of Naïve T Cells Is Associated with Aerobic Fitness and Energy Expenditure of Active and Inactive Adults. Med Sci Sports Exerc. 2022;54:1288–99. doi:10.1249/MSS.0000000000002914.
6. Mehta MM, Weinberg SE, Chandel NS. Mitochondrial control of immunity: beyond ATP. Nat Rev Immunol. 2017;17:608–20. doi:10.1038/nri.2017.66.
7. Angajala A, Lim S, Phillips JB, Kim J-H, Yates C, You Z, Tan M. Diverse Roles of Mitochondria in Immune Responses: Novel Insights Into Immuno-Metabolism. Front Immunol. 2018;9:1605. doi:10.3389/fimmu.2018.01605.
8. Rosa-Neto JC, Lira FS, Little JP, Landells G, Islam H, Chazaud B, et al. Immunometabolism-fit: How exercise and training can modify T cell and macrophage metabolism in health and disease. Exerc Immunol Rev. 2022;28:29–46.
9. Islam H. Recent advances in exercise immunometabolism: Immune cell bioenergetics, muscle-immune cell interactions, and potential dietary adjuvants. Curr Opin Clin Nutr Metab Care. 2025;28:469–76. doi:10.1097/MCO.0000000000001157.
10. Voss K, Hong HS, Bader JE, Sugiura A, Lyssiotis CA, Rathmell JC. A guide to interrogating immunometabolism. Nat Rev Immunol. 2021;21:637–52. doi:10.1038/s41577-021-00529-8.
11. Hodgman CF, Hunt RM, Crane JC, Elzayat MT, LaVoy EC. A Scoping Review on the Effects of Physical Exercise and Fitness on Peripheral Leukocyte Energy Metabolism in Humans. Exerc Immunol Rev. 2023;29:54–87.
12. Islam H, Tsai S-H, Figueiredo C, Jackson GS, Marcotte-Chénard A, Bosak J, et al. Direct assessment of leukocyte signalling and cytokine secretion reveals exercise intensity-dependent reductions in anti-inflammatory cytokine action. J Physiol. 2024;602:2717–36. doi:10.1113/JP286228.
13. Schlagheck ML, Walzik D, Joisten N, Koliamitra C, Hardt L, Metcalfe AJ, et al. Cellular immune response to acute exercise: Comparison of endurance and resistance exercise. Eur J Haematol. 2020;105:75–84. doi:10.1111/ejh.13412.
14. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nat Rev Immunol. 2011;11:607–15. doi:10.1038/nri3041.
15. Shaw DM, Merien F, Braakhuis A, Dulson D. T-cells and their cytokine production: The anti-inflammatory and immunosuppressive effects of strenuous exercise. Cytokine. 2018;104:136–42. doi:10.1016/j.cyto.2017.10.001.

16. Theall B, Stampley J, Cho E, Granger J, Johannsen NM, Irving BA, Spielmann G. Impact of acute exercise on peripheral blood mononuclear cells nutrient sensing and mitochondrial oxidative capacity in healthy young adults. Physiol Rep. 2021;9:e15147. doi:10.14814/phy2.15147.
17. Bhatti JS, Bhatti GK, Reddy PH. Mitochondrial dysfunction and oxidative stress in metabolic disorders - A step towards mitochondria based therapeutic strategies. Biochim Biophys Acta Mol Basis Dis. 2017;1863:1066–77. doi:10.1016/j.bbadis.2016.11.010.
18. Weyh C, Krüger K, Strasser B. Physical Activity and Diet Shape the Immune System during Aging. Nutrients 2020. doi:10.3390/nu12030622.
19. Chen W, Zhao H, Li Y. Mitochondrial dynamics in health and disease: mechanisms and potential targets. Signal Transduct Target Ther. 2023;8:333. doi:10.1038/s41392-023-01547-9.
20. Worbs T, Hammerschmidt SI, Förster R. Dendritic cell migration in health and disease. Nat Rev Immunol. 2017;17:30–48. doi:10.1038/nri.2016.116.
21. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. Immunity. 2013;38:633–43. doi:10.1016/j.immuni.2013.04.005.
22. Kaelin WG, McKnight SL. Influence of metabolism on epigenetics and disease. Cell. 2013;153:56–69. doi:10.1016/j.cell.2013.03.004.
23. Rambold AS, Pearce EL. Mitochondrial Dynamics at the Interface of Immune Cell Metabolism and Function. Trends Immunol. 2018;39:6–18. doi:10.1016/j.it.2017.08.006.
24. Chapman NM, Chi H. Metabolic adaptation of lymphocytes in immunity and disease. Immunity. 2022;55:14–30. doi:10.1016/j.immuni.2021.12.012.
25. Long L, Wei J, Lim SA, Raynor JL, Shi H, Connelly JP, et al. CRISPR screens unveil signal hubs for nutrient licensing of T cell immunity. Nature. 2021;600:308–13. doi:10.1038/s41586-021-04109-7.
26. Da Rosa PC, Bertomeu JB, Royes LFF, Osiecki R. The physical exercise-induced oxidative/inflammatory response in peripheral blood mononuclear cells: Signaling cellular energetic stress situations. Life Sci. 2023;321:121440. doi:10.1016/j.lfs.2023.121440.
27. Li B, Yang C, Jia G, Liu Y, Wang N, Yang F, et al. Comprehensive evaluation of the effects of long-term cryopreservation on peripheral blood mononuclear cells using flow cytometry. BMC Immunol. 2022;23:30. doi:10.1186/s12865-022-00505-4.
28. Canet F, Díaz-Pozo P, Luna-Marco C, Fernandez-Reyes M, Vezza T, Marti M, et al. Mitochondrial redox impairment and enhanced autophagy in peripheral blood mononuclear cells from type 1 diabetic patients. Redox Biol. 2022;58:102551. doi:10.1016/j.redox.2022.102551.
29. Faas MM, Vos P de. Mitochondrial function in immune cells in health and disease. Biochim Biophys Acta Mol Basis Dis. 2020;1866:165845. doi:10.1016/j.bbadis.2020.165845.
30. Halkias J, Melichar HJ, Taylor KT, Robey EA. Tracking migration during human T cell development. Cell Mol Life Sci. 2014;71:3101–17. doi:10.1007/s00018-014-1607-2.
31. Almeida L, Lochner M, Berod L, Sparwasser T. Metabolic pathways in T cell activation and lineage differentiation. Semin Immunol. 2016;28:514–24. doi:10.1016/j.smim.2016.10.009.

32. Almeida L, Dhillon-LaBrooy A, Carriche G, Berod L, Sparwasser T. CD4+ T-cell differentiation and function: Unifying glycolysis, fatty acid oxidation, polyamines NAD mitochondria. J Allergy Clin Immunol. 2021;148:16–32. doi:10.1016/j.jaci.2021.03.033.
33. van der Windt GJW, Pearce EL. Metabolic switching and fuel choice during T-cell differentiation and memory development. Immunol Rev. 2012;249:27–42. doi:10.1111/j.1600-065X.2012.01150.x.
34. Sun L, Su Y, Jiao A, Wang X, Zhang B. T cells in health and disease. Signal Transduct Target Ther. 2023;8:235. doi:10.1038/s41392-023-01471-y.
35. Heikkilä N, Hetemäki I, Sormunen S, Isoniemi H, Kekäläinen E, Saramäki J, Arstila TP. Peripheral differentiation patterns of human T cells. Eur J Immunol. 2022;52:882–94. doi:10.1002/eji.202149465.
36. Lanzavecchia A, Sallusto F. Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. Science. 2000;290:92–7. doi:10.1126/science.290.5489.92.
37. Gattinoni L, Speiser DE, Lichterfeld M, Bonini C. T memory stem cells in health and disease. Nat Med. 2017;23:18–27. doi:10.1038/nm.4241.
38. O'Sullivan D. The metabolic spectrum of memory T cells. Immunol Cell Biol. 2019;97:636–46. doi:10.1111/imcb.12274.
39. Koh C-H, Lee S, Kwak M, Kim B-S, Chung Y. CD8 T-cell subsets: heterogeneity, functions, and therapeutic potential. Exp Mol Med. 2023;55:2287–99. doi:10.1038/s12276-023-01105-x.
40. Ma S, Ming Y, Wu J, Cui G. Cellular metabolism regulates the differentiation and function of T-cell subsets. Cell Mol Immunol. 2024;21:419–35. doi:10.1038/s41423-024-01148-8.
41. Plitas G, Rudensky AY. Regulatory T Cells: Differentiation and Function. Cancer Immunol Res. 2016;4:721–5. doi:10.1158/2326-6066.CIR-16-0193.
42. Hori S. FOXP3 as a master regulator of Treg cells. Nat Rev Immunol. 2021;21:618–9. doi:10.1038/s41577-021-00598-9.
43. Long M, Park S-G, Strickland I, Hayden MS, Ghosh S. Nuclear factor-kappaB modulates regulatory T cell development by directly regulating expression of Foxp3 transcription factor. Immunity. 2009;31:921–31. doi:10.1016/j.immuni.2009.09.022.
44. Khantakova JN, Bulygin AS, Sennikov SV. The Regulatory-T-Cell Memory Phenotype: What We Know. Cells 2022. doi:10.3390/cells11101687.
45. Rosenblum MD, Way SS, Abbas AK. Regulatory T cell memory. Nat Rev Immunol. 2016;16:90–101. doi:10.1038/nri.2015.1.
46. Mills EL, Kelly B, O'Neill LAJ. Mitochondria are the powerhouses of immunity. Nat Immunol. 2017;18:488–98. doi:10.1038/ni.3704.
47. Hu T, Liu C-H, Lei M, Zeng Q, Li L, Tang H, Zhang N. Metabolic regulation of the immune system in health and diseases: mechanisms and interventions. Signal Transduct Target Ther. 2024;9:268. doi:10.1038/s41392-024-01954-6.
48. Bennett CF, Latorre-Muro P, Puigserver P. Mechanisms of mitochondrial respiratory adaptation. Nat Rev Mol Cell Biol. 2022;23:817–35. doi:10.1038/s41580-022-00506-6.
49. Yin M, O'Neill LAJ. The role of the electron transport chain in immunity. FASEB J. 2021;35:e21974. doi:10.1096/fj.202101161R.

50. Chang C-H, Curtis JD, Maggi LB, Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell. 2013;153:1239–51. doi:10.1016/j.cell.2013.05.016.
51. Divakaruni AS, Jastroch M. A practical guide for the analysis, standardization and interpretation of oxygen consumption measurements. Nat Metab. 2022;4:978–94. doi:10.1038/s42255-022-00619-4.
52. Gnaiger E. Mitochondrial Pathways and Respiratory Control: Bioenergetics Communications; 2020.
53. Gnaiger E, Aasander Frostner E, Abdul Karim N, Abdel-Rahman EA, Abumrad NA, Acuna-Castroviejo D, et al. Mitochondrial physiology: Bioenergetics Communications; 2020.
54. Liu Y, Fu T, Li G, Li B, Luo G, Li N, Geng Q. Mitochondrial transfer between cell crosstalk - An emerging role in mitochondrial quality control. Ageing Res Rev. 2023;91:102038. doi:10.1016/j.arr.2023.102038.
55. van Horssen J, van Schaik P, Witte M. Inflammation and mitochondrial dysfunction: A vicious circle in neurodegenerative disorders? Neurosci Lett. 2019;710:132931. doi:10.1016/j.neulet.2017.06.050.
56. Xu X, Pang Y, Fan X. Mitochondria in oxidative stress, inflammation and aging: from mechanisms to therapeutic advances. Signal Transduct Target Ther. 2025;10:190. doi:10.1038/s41392-025-02253-4.
57. Magnani ND, Marchini T, Calabró V, Alvarez S, Evelson P. Role of Mitochondria in the Redox Signaling Network and Its Outcomes in High Impact Inflammatory Syndromes. Front Endocrinol (Lausanne). 2020;11:568305. doi:10.3389/fendo.2020.568305.
58. Marchi S, Guilbaud E, Tait SWG, Yamazaki T, Galluzzi L. Mitochondrial control of inflammation. Nat Rev Immunol. 2023;23:159–73. doi:10.1038/s41577-022-00760-x.
59. Contrepois K, Wu S, Moneghetti KJ, Hornburg D, Ahadi S, Tsai M-S, et al. Molecular Choreography of Acute Exercise. Cell. 2020;181:1112-1130.e16. doi:10.1016/j.cell.2020.04.043.
60. Simpson RJ, Boßlau TK, Weyh C, Niemiro GM, Batatinha H, Smith KA, Krüger K. Exercise and adrenergic regulation of immunity. Brain Behav Immun. 2021;97:303–18. doi:10.1016/j.bbi.2021.07.010.
61. Da Scheffer DL, Latini A. Exercise-induced immune system response: Anti-inflammatory status on peripheral and central organs. Biochim Biophys Acta Mol Basis Dis. 2020;1866:165823. doi:10.1016/j.bbadis.2020.165823.
62. Campbell JP, Turner JE. Debunking the Myth of Exercise-Induced Immune Suppression: Redefining the Impact of Exercise on Immunological Health Across the Lifespan. Front Immunol. 2018;9:648. doi:10.3389/fimmu.2018.00648.
63. Risøy BA, Raastad T, Hallén J, Lappegård KT, Baeverfjord K, Kravdal A, et al. Delayed leukocytosis after hard strength and endurance exercise: aspects of regulatory mechanisms. BMC Physiol. 2003;3:14. doi:10.1186/1472-6793-3-14.
64. Zúñiga TM, Baker FL, Smith KA, Batatinha H, Lau B, Burgess SC, et al. Clonal Kinetics and Single-Cell Transcriptional Profiles of T Cells Mobilized to Blood by Acute Exercise. Med Sci Sports Exerc. 2023;55:991–1002. doi:10.1249/MSS.0000000000003130.
65. Hunt RM, Elzayat MT, Markofski MM, Laughlin M, LaVoy EC. Characterization of transitional memory CD4+ and CD8+ T-cell mobilization during and after an acute

bout of exercise. Front Sports Act Living. 2023;5:1120454. doi:10.3389/fspor.2023.1120454).
66. Margaritelis NV, Theodorou AA, Baltzopoulos V, Maganaris CN, Paschalis V, Kyparos A, Nikolaidis MG. Muscle damage and inflammation after eccentric exercise: can the repeated bout effect be removed? Physiol Rep 2015. doi:10.14814/phy2.12648.
67. Palmowski J, Gebhardt K, Reichel T, Frech T, Ringseis R, Eder K, et al. The Impact of Exercise Serum on Selected Parameters of CD4+ T Cell Metabolism. Immuno. 2021;1:119–31. doi:10.3390/immuno1030008.
68. Siedlik JA, Deckert JA, Benedict SH, Bhatta A, Dunbar AJ, Vardiman JP, Gallagher PM. T cell activation and proliferation following acute exercise in human subjects is altered by storage conditions and mitogen selection. J Immunol Methods. 2017;446:7–14. doi:10.1016/j.jim.2017.03.017.
69. Holbrook AK, Peterson HD, Bianchi SA, Macdonald BW, Bredahl EC, Belshan M, Siedlik JA. CD4+ T cell activation and associated susceptibility to HIV-1 infection in vitro increased following acute resistance exercise in human subjects. Physiol Rep. 2019;7:e14234. doi:10.14814/phy2.14234.
70. Padilha CS, Ah Morano AE von, Krüger K, Rosa-Neto JC, Lira FS. The growing field of immunometabolism and exercise: Key findings in the last 5 years. J Cell Physiol. 2022;237:4001–20. doi:10.1002/jcp.30866.
71. Spielmann G, McFarlin BK, O'Connor DP, Smith PJW, Pircher H, Simpson RJ. Aerobic fitness is associated with lower proportions of senescent blood T-cells in man. Brain Behav Immun. 2011;25:1521–9. doi:10.1016/j.bbi.2011.07.226.
72. Franklin BA, Wedig IJ, Sallis RE, Lavie CJ, Elmer SJ. Physical Activity and Cardiorespiratory Fitness as Modulators of Health Outcomes: A Compelling Research-Based Case Presented to the Medical Community. Mayo Clin Proc. 2023;98:316–31. doi:10.1016/j.mayocp.2022.09.011.
73. Zeiher J, Ombrellaro KJ, Perumal N, Keil T, Mensink GBM, Finger JD. Correlates and Determinants of Cardiorespiratory Fitness in Adults: a Systematic Review. Sports Med Open. 2019;5:39. doi:10.1186/s40798-019-0211-2;
74. Lavie CJ, Ozemek C, Carbone S, Katzmarzyk PT, Blair SN. Sedentary Behavior, Exercise, and Cardiovascular Health. Circ Res. 2019;124:799–815. doi:10.1161/CIRCRESAHA.118.312669.
75. Dorneles GP, Da Silva I, Boeira MC, Valentini D, Fonseca SG, Dal Lago P, et al. Cardiorespiratory fitness modulates the proportions of monocytes and T helper subsets in lean and obese men. Scand J Med Sci Sports. 2019;29:1755–65. doi:10.1111/sms.13506.
76. Proschinger S, Winker M, Joisten N, Bloch W, Palmowski J, Zimmer P. The effect of exercise on regulatory T cells: A systematic review of human and animal studies with future perspectives and methodological recommendations. Exerc Immunol Rev. 2021;27:142–66.
77. Antunes BM, Rosa-Neto JC, Batatinha HAP, Franchini E, Teixeira AM, Lira FS. Physical fitness status modulates the inflammatory proteins in peripheral blood and circulating monocytes: role of PPAR-gamma. Sci Rep. 2020;10:14094. doi:10.1038/s41598-020-70731-6.

78. Yousefzadeh MJ, Flores RR, Zhu Y, Schmiechen ZC, Brooks RW, Trussoni CE, et al. An aged immune system drives senescence and ageing of solid organs. Nature. 2021;594:100–5. doi:10.1038/s41586-021-03547-7.
79. Xia T, Zhou Y, An J, Cui Z, Zhong X, Cui T, et al. Benefit delayed immunosenescence by regulating CD4+T cells: A promising therapeutic target for aging-related diseases. Aging Cell. 2024;23:e14317. doi:10.1111/acel.14317.
80. Wikby A, Månsson IA, Johansson B, Strindhall J, Nilsson SE. The immune risk profile is associated with age and gender: findings from three Swedish population studies of individuals 20-100 years of age. Biogerontology. 2008;9:299–308. doi:10.1007/s10522-008-9138-6.
81. Pawelec G, Bronikowski A, Cunnane SC, Ferrucci L, Franceschi C, Fülöp T, et al. The conundrum of human immune system "senescence". Mech Ageing Dev. 2020;192:111357. doi:10.1016/j.mad.2020.111357.
82. Shirakawa K, Sano M. T Cell Immunosenescence in Aging, Obesity, and Cardiovascular Disease. Cells 2021. doi:10.3390/cells10092435.
83. Miwa S, Kashyap S, Chini E, Zglinicki T von. Mitochondrial dysfunction in cell senescence and aging. J Clin Invest 2022. doi:10.1172/JCI158447.
84. Silaidos CV, Reutzel M, Wachter L, Dieter F, Ludin N, Blum WF, et al. Age-related changes in energy metabolism in peripheral mononuclear blood cells (PBMCs) and the brains of cognitively healthy seniors. Geroscience. 2024;46:981–98. doi:10.1007/s11357-023-00810-9.
85. San-Millán I. The Key Role of Mitochondrial Function in Health and Disease. Antioxidants (Basel) 2023. doi:10.3390/antiox12040782.
86. Morales-Rosales SL, Rivero-Segura NA, Königsberg M. Mitochondrial Function in Aging. In: Gomez-Verjan JC, Rivero-Segura NA, editors. Clinical Genetics and Genomics of Aging. Cham: Springer International Publishing; 2020. p. 65–85. doi:10.1007/978-3-030-40955-5_4.
87. Sellami M, Gasmi M, Denham J, Hayes LD, Stratton D, Padulo J, Bragazzi N. Effects of Acute and Chronic Exercise on Immunological Parameters in the Elderly Aged: Can Physical Activity Counteract the Effects of Aging? Front Immunol. 2018;9:2187. doi:10.3389/fimmu.2018.02187.
88. Kozlov AV, Lancaster JR, Meszaros AT, Weidinger A. Mitochondria-meditated pathways of organ failure upon inflammation. Redox Biol. 2017;13:170–81. doi:10.1016/j.redox.2017.05.017.
89. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011;469:221–5. doi:10.1038/nature09663.
90. Casanova A, Wevers A, Navarro-Ledesma S, Pruimboom L. Mitochondria: It is all about energy. Front Physiol. 2023;14:1114231. doi:10.3389/fphys.2023.1114231.
91. Gebhardt K, Hebecker A, Honekamp C, Nolte S, Barthkuhn M, Wilhelm J, et al. Respiratory and Metabolic Responses of CD4 + T Cells to Acute Exercise and Their Association with Cardiorespiratory Fitness. Med Sci Sports Exerc. 2024;56:1882–92. doi:10.1249/MSS.0000000000003483.
92. Gebhardt K, Hebecker A, Sommer N, Ringseis R, Eder K, Huber M, et al. PBMCs Mitochondrial Respiration and Its Relation to Immunity, Fitness, and Metabolic Risk in the Healthy Elderly. J Cell Physiol. 2025;240:e70096. doi:10.1002/jcp.70096.

93. Gebhardt K, Krüger K. Effects of Cardiorespiratory Fitness on Immune Cell Mitochondrial Metabolism in Health and Disease. DtschZSportmed. 2024;75:123–31. doi:10.5960/dzsm.2024.587.
94. Drake JC, Wilson RJ, Yan Z. Molecular mechanisms for mitochondrial adaptation to exercise training in skeletal muscle. FASEB J. 2015;30:13–22. doi:10.1096/fj.15-276337.
95. Nedel W, Strogulski NR, Kopczynski A, Portela LV. Assessment of mitochondrial function and its prognostic role in sepsis: a literature review. Intensive Care Med Exp. 2024;12:107. doi:10.1186/s40635-024-00694-9.
96. Rose S, Carvalho E, Diaz EC, Cotter M, Bennuri SC, Azhar G, et al. A comparative study of mitochondrial respiration in circulating blood cells and skeletal muscle fibers in women. Am J Physiol Endocrinol Metab. 2019;317:E503-E512. doi:10.1152/ajpendo.00084.2019.
97. Hedges CP, Woodhead JST, Wang HW, Mitchell CJ, Cameron-Smith D, Hickey AJR, Merry TL. Peripheral blood mononuclear cells do not reflect skeletal muscle mitochondrial function or adaptation to high-intensity interval training in healthy young men. J Appl Physiol (1985). 2019;126:454–61. doi:10.1152/japplphysiol.00777.2018.
98. Westerlund E, Marelsson SE, Karlsson M, Sjövall F, Chamkha I, Åsander Frostner E, et al. Correlation of mitochondrial respiration in platelets, peripheral blood mononuclear cells and muscle fibers. Heliyon. 2024;10:e26745. doi:10.1016/j.heliyon.2024.e26745.
99. Westermann S, Bennühr BC, Fumo AR, Rohm M, Hiller K. Muscle-immune metabolic crosstalk: shared pathways in cachexia and exercise. Curr Opin Biotechnol. 2026;98:103455. doi:10.1016/j.copbio.2026.103455.
100. Walzik D, Joisten N, Metcalfe AJ, Proschinger S, Schenk A, Wenzel C, et al. Acute exercise rewires the proteomic landscape of human immune cells. Nat Commun. 2026;17:130. doi:10.1038/s41467-025-68101-9.
101. Geltink RIK, Kyle RL, Pearce EL. Unraveling the Complex Interplay Between T Cell Metabolism and Function. Annu Rev Immunol. 2018;36:461–88. doi:10.1146/annurev-immunol-042617-053019.
102. Tsai H-H, Chang S-C, Chou C-H, Weng T-P, Hsu C-C, Wang J-S. Exercise Training Alleviates Hypoxia-induced Mitochondrial Dysfunction in the Lymphocytes of Sedentary Males. Sci Rep. 2016;6:35170. doi:10.1038/srep35170.
103. van den Bossche J, O'Neill LA, Menon D. Macrophage Immunometabolism: Where Are We (Going)? Trends Immunol. 2017;38:395–406. doi:10.1016/j.it.2017.03.001.
104. Dominelli PB, Wiggins CC, Roy TK, Secomb TW, Curry TB, Joyner MJ. The Oxygen Cascade During Exercise in Health and Disease. Mayo Clin Proc. 2021;96:1017–32. doi:10.1016/j.mayocp.2020.06.063.
105. DeConne TM, Muñoz ER, Sanjana F, Hobson JC, Martens CR. Cardiometabolic risk factors are associated with immune cell mitochondrial respiration in humans. Am J Physiol Heart Circ Physiol. 2020;319:H481-H487. doi:10.1152/ajpheart.00434.2020.
106. Sánchez J, Picó C, Ahrens W, Foraita R, Fraterman A, Moreno LA, et al. Transcriptome analysis in blood cells from children reveals potential early biomarkers of metabolic alterations. Int J Obes (Lond). 2017;41:1481–8. doi:10.1038/ijo.2017.132.

107. Yuan Q, Zeng ZL, Yang S, Li A, Zu X, Liu J. Mitochondrial Stress in Metabolic Inflammation: Modest Benefits and Full Losses. Oxid Med Cell Longev. 2022;2022:8803404. doi:10.1155/2022/8803404.
108. Yang Y, Zeng Y, Liu Z, Huang J-A. Pathogen-induced mitochondrial dysfunction: mechanistic insights, immune crosstalk, and therapeutic opportunities. Front Cell Infect Microbiol. 2025;15:1714998. doi:10.3389/fcimb.2025.1714998.
109. Wen H, Deng H, Li B, Chen J, Zhu J, Zhang X, et al. Mitochondrial diseases: from molecular mechanisms to therapeutic advances. Signal Transduct Target Ther. 2025;10:9. doi:10.1038/s41392-024-02044-3.
110. Feng Z, Tan Z, Lu D. Mitochondrial bioenergetics dysfunction in T2DM: linking oxidative stress to insulin resistance. Front Endocrinol (Lausanne). 2025;16:1674477. doi:10.3389/fendo.2025.1674477.
111. Ehinger JK, Westerlund E, Frostner EÅ, Karlsson M, Paul G, Sjövall F, Elmér E. Mitochondrial function in peripheral blood cells across the human lifespan. NPJ Aging. 2024;10:10. doi:10.1038/s41514-023-00130-4.
112. Weyh C, Bauer P, Größer VW, Zhupani D, Cemic F, Huber M, et al. Immunological biomarkers and molecular signatures in healthy aging: early dynamics of subclinical atherosclerosis in a homogeneous elderly cohort. BMC Cardiovasc Disord. 2025;25:736. doi:10.1186/s12872-025-05206-5.
113. Bharath LP, Agrawal M, McCambridge G, Nicholas DA, Hasturk H, Liu J, et al. Metformin Enhances Autophagy and Normalizes Mitochondrial Function to Alleviate Aging-Associated Inflammation. Cell Metab. 2020;32:44-55.e6. doi:10.1016/j.cmet.2020.04.015.
114. Bencivenga L, Strumia M, Rolland Y, Martinez L, Cestac P, Guyonnet S, et al. Biomarkers of mitochondrial dysfunction and inflammaging in older adults and blood pressure variability. Geroscience. 2023;45:797–809. doi:10.1007/s11357-022-00697-y.
115. Santoro A, Martucci M, Conte M, Capri M, Franceschi C, Salvioli S. Inflammaging, hormesis and the rationale for anti-aging strategies. Ageing Res Rev. 2020;64:101142. doi:10.1016/j.arr.2020.101142.
116. Peng S-C, Lin Y-T, Hsu C-C, Fu T-C, Wang J-S. Effects of concentric and eccentric exercise regimens on bioenergetic efficiency of lymphocytes in sedentary males. Eur J Sport Sci. 2024;24:1830–43. doi:10.1002/ejsc.12221.
117. Andonian BJ, Koss A, Koves TR, Hauser ER, Hubal MJ, Pober DM, et al. Rheumatoid arthritis T cell and muscle oxidative metabolism associate with exercise-induced changes in cardiorespiratory fitness. Sci Rep. 2022;12:7450. doi:10.1038/s41598-022-11458-4.
118. Dias RG, Silva MSM, Duarte NE, Bolani W, Alves CR, Junior JRL, et al. PBMCs express a transcriptome signature predictor of oxygen uptake responsiveness to endurance exercise training in men. Physiol Genomics. 2015;47:13–23. doi:10.1152/physiolgenomics.00072.2014.
119. Ozemek C, Laddu DR, Lavie CJ, Claeys H, Kaminsky LA, Ross R, et al. An Update on the Role of Cardiorespiratory Fitness, Structured Exercise and Lifestyle Physical Activity in Preventing Cardiovascular Disease and Health Risk. Prog Cardiovasc Dis. 2018;61:484–90. doi:10.1016/j.pcad.2018.11.005.

120. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell. 2013;153:1194–217. doi:10.1016/j.cell.2013.05.039.
121. Monzel AS, Enríquez JA, Picard M. Multifaceted mitochondria: moving mitochondrial science beyond function and dysfunction. Nat Metab. 2023;5:546–62. doi:10.1038/s42255-023-00783-1.
122. Argüello RJ, Combes AJ, Char R, Gigan J-P, Baaziz AI, Bousiquot E, et al. SCENITH: A Flow Cytometry-Based Method to Functionally Profile Energy Metabolism with Single-Cell Resolution. Cell Metab. 2020;32:1063-1075.e7. doi:10.1016/j.cmet.2020.11.007.

Formal Declaration

"I hereby declare that I have prepared the thesis at hand independently and without undue aid or the use of any resources other than indicated within the thesis. All parts of my thesis taken either verbatim or analogously from the published or unpublished works of or based on oral communications with others are indicated as such. Regarding all aspects of my scientific enquiries as they appear in my thesis, I have upheld the tenets of good scientific practice as laid out in the "Satzung der Justus-Liebig-Universität Gießen zur Sicherung guter wissenschaftlicher Praxis" and complied with the precept of ethics, data protection and animal welfare. I declare that I have neither directly nor indirectly given monetary or any other valuable considerations to others in connection with the thesis at hand. I declare that I have not presented the thesis at hand, either in an identical or similar form, to an examination office or agency in Germany or any other country as part of any examination or degree. All materials from other sources as well as all works performed by others used or directly referenced within the thesis at hand have been indicated as such. In particular, all persons involved directly or indirectly in the development of the thesis at hand have been named. I agree with the screening of my thesis for plagiarism via offline or online detection-software."

Place, Date

Signature

Appendix

A: Supplementary Materials from Gebhardt et al (2024)

Gebhardt K, Hebecker A, Honekamp C, Nolte S, Barthkuhn M, Wilhelm J, Klatt S, Weyh C, Sommer N, and Krüger K (2024). Respiratory and Metabolic Responses of CD4+ T Cells to Acute Exercise and Their Association with Cardiorespiratory Fitness. Medicine & Science in Sports & Exercise, 56(10), 1882-1892.

Full text available at: https://doi.org/10.1249/MSS.0000000000003483

Supplementary: http://links.lww.com/MSS/D42

B: Supplementary Materials from Gebhardt et al (2025)

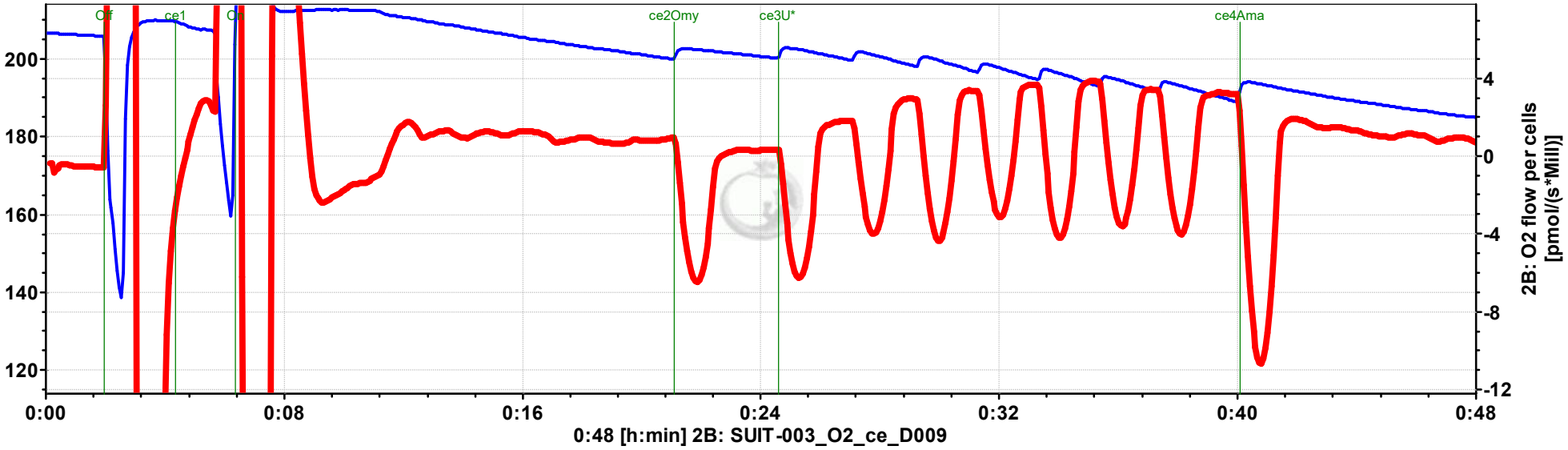


Figure S1. Typical trace from intact peripheral blood mononuclear cells (PBMCs) from older participants using Oxygraph-2k. The SUIT protocol consisted of sequential titration of multiple substrates (left y-axis, oxygen concentration in the chamber; right y-axis, oxygen flux; x-axis, time in h and min; ce2Omy, ATP synthase inhibitor Oligomycin, ce3U*, uncoupling agent FCCP, ce4Ama, complex III inhibitor antimycin A). The red line represents the cell-specific O2 flow, calculated as the negative time derivative of the O2 concentration, expressed as pmol/s/10^6 cells.

Table S1. Cytokine ranges and Detection rates.

Marker	Standard Curve (pg/mL)	Detection rate
Adiponectin/Acrp30	823-200000	100%
CCL4/MIP-1beta	123 - 30000	100%
CXCL9/MIG	617 - 150000	100%
CXCL13/BLC/BCA-1	18.5 - 4500	100%
Galectin-3	16.9 - 4100	100%
GDF-15	18.5 - 4500	100%
ICAM-1/CD54	7000 - 1700000	100%
IFN-gamma	2.67 - 650	86%
IL-1ra/IL-1F3	28.8 - 7000	98%
IL-6	3.25 - 790	98%
IL-7	5.14 - 1250	100%
IL8/CXCL8	4.12 - 1000	100%
IL-10	1.89 - 460	100%
IL-15	6.3 - 1550	100%
IL-17/IL17A	13.2 - 3200	100%
IL-18/IL-1F4	13.9 - 3380	100%
MMP-2	276 - 67000	100%
MMP-9	123 - 30000	100%
Myeloperoxidase/MPO	123 - 30000	100%
Myoglobin	15.4 - 3800	100%
Tenascin C	53.5 - 13000	100%
TNF-alpha	8.23 - 2000	100%
VEGF	11.5 - 2800	100%

Table S2. Absolute values of serum cytokines.

Cytokine	MW (SD)	Minimum	Maximum
IL1-ra	408.40 (120.82)	230.77	711.54
IL-6	1.77 (3.10)	0.11	12.19
IL-7	11.25 (5.36)	5.73	23.37
IL-8	6.15 (3.88)	1.29	14.16
IL-10	0.86 (0.50)	0.31	2.27
IL-15	4.60 (4.08)	1.78	16.38
IL-17	1.21 (1.06)	0.59	5.08
IL-18	4735.76 (1938.06)	2034.34	8894.73
IFN-gamma	45.60 (77.55)	0.84	286.55
TNF-alpha	3.96 (1.21)	2.48	6.56
ICAM1	99,404 (74,176.96)	11562.22	279,676.03
VEGF	85.02 (66.67)	7.66	271.88
Adiponectin	7.67 (1.76)	5.45	11.40
Tenascin-C	2.49 (0.79)	1.08	3.75
Galectin	3.25 (0.71)	2.02	4.23
MMP2	195.60 (16.76)	152.56	218.68
MMP9	168.96 (81.94)	55.75	316.67
MPO	72.96 (30.49)	23.22	114.02
Myoglobin	4.44 (1.59)	2.70	8.69
CCL4	123.80 (32.39)	83.64	191.19
CXCL9	452.96 (225.48)	300.32	1030.46
CXCL13	31.24 (18.93)	3.75	68.52
GDF15	13,126.21 (4315.77)	5905.45	24,511.87

Table S3: Multivariate regression model

	Model 1			Model 2			Model 3		
	Adj. R^2 (p-value)	*ß (95% CI)*	*p-value*	*Adj. R^2 (p-value)*	*ß (95% CI)*	*p-value*	*Adj. R^2 (p-value)*	*ß (95% CI)*	*p-value*
CD4 - basal	**0.436 (0.005)**	**0.081 (0.012 – 0.150)**	**0.025**	**0.416 (0.014)**	**0.083 (0.012 – 0.154)**	**0.026**	**0.378 (0.035)**	**0.082 (0.007 – 0.156)**	**0.034**
Age		**0.229 (0.042-0.417)**	**0.020**		**0.219 (0.024 – 0.414)**	**0.030**		**0.233 (0.016 – 0.449)**	**0.037**
Body fat (%)					-0.036 (-0.145 – 0.74)	0.497		-0.062 (-0.248 – 0.123)	0.480
VO_{2peak}								-0.026 (-0.167 – 0.115)	0.698
CD4 - maximal	0.218 (0.062)	**0.174 (0.021 – 0.327)**	**0.028**	0.228 (0.087)	0.168 (0.014 – 0.321)	0.035	0.228 (0.119)	0.175 (0.019 – 0.330)	0.030
Age		0.109 (-0.305 – 0.522)	0.584		0.144 (-0.275 – 0.564)	0.472		0.071 (-0.381 – 0.523)	0.740
Body fat (%)					0.120 (-0.115 – 0.355)	0.291		0.261 (-0.126 – 0.648)	0.169
VO_{2peak}								0.135 (-0.159 – 0.249)	0.338
CD4 naive - maximal	**0.253 (0.044)**	**0.119 (0.022 – 0.216)**	**0.019**	0.276 (0.058)	0.116 (0.020 – 0.212)	**0.021**	0.221 (0.125)	0.115 (0.011 – 0.218)	0.032
Age		0.147 (-0.254 – 0.548)	0.445		0.184 (-0.219 – 0.586)	0.344		0.174 (-0.273 – 0.620)	0.415
Body fat (%)					0.129 (-0.098 – 0.356)	0.244		0.150 (-0.249 – 0.548)	0.432
VO_{2peak}								0.020 (- 0.283 – 0.322)	0.891
CD4 EM - maximal	0.185 (0.084)	-0.171 (-0.334 – -0.009)	0.040	**0.309 (0.043)**	**-0.200(-0.353 – -0.046)**	**0.015**	0.277 (0.083)	-0.197 (-0.356 – -0.038)	0.019
Age		0.184 (-0.235 – 0.602)	0.364		0.241 (-0.152 – 0.634)	0.209		0.198 (0.-0.233 – 0.629)	0.339
Body fat (%)					0.203 (-0.024 – 0.429)	0.075		0.288 (-0.087 – 0.664)	0.121
VO_{2peak}								0.082 (-0.201 – 0.365)	0.542
CD8 - maximal	0.172 (0.095)	0.265 (0.006 – 0.525)	0.046	0.149 (0.162)	0.243 (-0.029 – 0.515)	0.076	0.122 (0.235)	0.246 (-0.033 – 0.524)	0.079

Age	0.184 (-0.237 – 0.366 0.605)		0.208 (-0.227 – 0.644)	0.322	0.151 (-0.323 – 0.625)	0.503
Body fat (%)			0.090 (-0.162 – 0.343)	0.455	0.205 (-0.210 – 0.620)	0.306
VO_{2peak}					0.110 (-0.202 – 0.422)	0.460

	Model 1			**Model 2**			**Model 3**		
	Adj. R² (p-value)	*ß (95% CI)*	*p-value*	*Adj. R² (p-value)*	*ß (95% CI)*	*p-value*	*Adj. R² (p-value)*	*ß (95% CI)*	*p-value*
IL-8 - maximal	**0.259 (0.041)**	**0.481 (0.095 – 0.867)**	**0.018**	0.207 (0.104)	0.467 (0.017 – 0.917)	0.043	0.308 (0.065)	0.596 (0.143 – 1.048)	0.014
Age		0.069 (-0.338 – 0.477)	0.721		0.078 (-0.363 – 0.518)	0.711		-0.084 (-0.545 – 0.376)	0.698
Body fat (%)					0.018 (-0.248 – 0.284)	0.885		0.234 (-0.132 – 0.600)	0.191
VO_{2peak}								0.238 (-0.057 – 0.534)	0.105
IL-17 - basal	**0.360 (0.014)**	-0,666 (-1.406 – 0.074)	0.074	0.316 (0.040)	-0.689 (-1.506 – 0.128)	0.092	0.263 (0.092)	-0.690 (-1.598 – 0.218)	0.125
Age		**0.207 (0.001 – 0.413)**	**0.05**		0.208 (-0.007 – 0.423)	0.057		0.208 (-0.041 – 0.456)	0.094
Body fat (%)					0.011 (-0.114 – 0.135)	0.858		0.011 (-0.216 – 0.238)	0.918
VO_{2peak}								0.001 (-0.162 - 0.163)	0.995
TNF-α - basal	**0.450 (0.004)**	**-0.703 (-1.279 – -0.127)**	**0.020**	**0.418 (0.014)**	**-0.743 (-1.378 – -0.109)**	**0.025**	**0.416 (0.024)**	**-0.780 (-1.425 – -0.135)**	**0.022**
Age		**0.253 (0.069 – 0.436)**	**0.010**		**0.258 (0.066 – 0.451)**	**0.012**		**0.291 (0.084 – 0.498)**	**0.009**
Body fat (%)					0.021 (-0.094 – 0.137)	0.696		-0.042 (-0.222 – 0.139)	0.628
VO_{2peak}								-0.062 (-0.199 – 0.075)	0.343
ICAM-1 - maximal	**0.247 (0.047)**	**0.000025 (0.000004 – 0.000046)**	**0.020**	0.285 (0.054)	0,000025 (0.000005 – 0.000045)	0.019	0.238 (0.111)	0,000027 (0.000003 - 0,000050)	0.028
Age		0.098 (-0.308 – 0.505)	0.614		0.138 (-0.266 – 0.541)	0.477		0.161 (-0.280 – 0.603)	0.445
Body fat (%)					0.141 (-0.085 – 0.366)	0.201		0.083 (-0.328 – 0.493)	0.670
VO_{2peak}								-0-055 (-0.376 – 0.266)	0.716

VEGF maximal	-	0.233 (0.054)	0.027 (0.004 – 0.05)	0.024	0.190 (0.119)	0.025 (0.000 – 0.050)	0.051	0.187 (0.157)	0.026 (0.001 – 0.052)	0.044
Age			0.168 (-0.238 – 0.573)	0.391		0.183 (-0.242 – 0.609)	0.371		0.111 (-0.348 – 0.569)	0.611
Body fat (%)						0.054 (-0.202 – 0.310)	0.660		0.192 (-0.209 – 0.593)	0.319
VO_{2peak}									-0.055 (-0.166 – 0.438)	0.716
Myoglobin basal	-	0.235 (0.052)	0.251 (-0.421 – 0.932)	0.438	0.189 (0.120)	0.247 (-0.450 – 0.943)	0.460	0.164 (0.182)	0.313 (-0.423 – 1.048)	0.376
Age			0.193 (-0.090 – 0.475)	0.167		0.187 (-0.107 – 0.481)	0.193		0.201 (-0.102 – 0.504)	0.176
Body fat (%)						-0.023 (-0.151 – 0,105)	0.707		-0.085 (-0.303 – 0.133)	0.416
VO_{2peak}									-0.060 (-0.227 – 0.108)	0.457

		Model 1			**Model 2**			**Model 3**		
		Adj. R^2 (p-value)	*ß (95% CI)*	*p-value*	*Adj. R^2 (p-value)*	*ß (95% CI)*	*p-value*	*Adj. R^2 (p-value)*	*ß (95% CI)*	*p-value*
STAT3 maximal	-	**0.415 (0.028)**	**5.994 (1.788 10.201)**	**0.010**	**0.473 (0.033)**	**4.716 (0.199 – 9.232)**	**0.042**	**0.495 (0.047)**	**4.992 (0.450 9.534)**	**0.035**
Age			-0.052 (-0.704 0.601)	0.864		0.111 (-0.567 – 0.788	0.720		-0.082 (-0.858 0.693)	0.812
Body fat (%)						0.177 (-0.099 – 0.453	0.181		0.325 (-0.076 0.726)	0.098
VO_{2peak}									0.155 (-0.149 0.459)	0.274
Glucose basal	-	**0.380 (0.011)**	0.078 (-0.002 0.159)	0.056	**0.358 (0.028)**	0.080 (-0.003 – 0.163	0.057	**0.373 (0.037)**	**0.092 (0.007 0.177)**	**0.036**
Age			0.194 (-0.013 0.400)	0.064		0.183 (-0.032 – 0.398	0.090		0.215 (-0.006 0.436)	0.056
Body fat (%)						-0.033 (-0.148– 0.082	0.547		-0.119(-0.311 0.073)	0.203
VO_{2peak}									-0.081 (-0.226 0.064)	0.251
Glucose maximal	–	0.214 (0.065)	0.192 (-0.022 0.362)	0.029	0.227 (0.089)	0.185 (0.015 – 0.355)	0.035	0.169 (0.177)	0.182 (-0.002 0.365)	0.052

Age		0.012 (-0.423 0.447)	0.955		0.052 (-0.389 – 0.493	0.805		0.042 (-0.435 0.519	0.852
Body fat (%)					0.123 (-0.112 – 0.358	0.282		0.149 (-0.265 0.562)	0.452
VO_{2peak}								0.024 (-0.289 0.337)	0.869
HbA1c – maximal	0.174 (0.093)	-5.736 (- 11,317 -0.156)	0.045	**0.310 (0.043)**	**-6.865 (-12.141 – -1.589)**	**0.014**	0.262 (0.093)	-6.729 (-12.309 1.148)	0.022
Age		0.167 (-0.254 0.588)	0.410		0.224 (-0.168 – 0.616	0.214		0.202 (-0.233 0.638)	0.334
Body fat (%)					0.211 (-0.017 – 0.438	0.067		0.252 (-0.126 0.630)	0.173
VO_{2peak}								0.041 (-0.249 0.331)	0.304

C: Supplementary Materials from Gebhardt et al (2024)

Extended Abstract

Gebhardt K[1], Krüger K[1]

Effekte der kardiorespiratorischen Fitness auf den mitochondrialen Immunzellmetabolismus in Gesundheit und Krankheit

Effects of Cardiorespiratory Fitness on Immune Cell Mitochondrial Metabolism in Health and Disease

ERWEITERTES ABSTRACT

ACCEPTED: January 2024

PUBLISHED ONLINE: June 2024

Gebhardt K, Krüger K. Effects of cardiorespiratory fitness on immune cell mitochondrial metabolism in health and disease. Dtsch Z Sportmed. 2024; 75: 123-131. doi:10.5960/dzsm.2024.587

1. JUSTUS LIEBIG UNIVERSITY GIESSEN, *Institute of Sports Science, Department of Exercise Physiology and Sports Therapy, Giessen, Germany*

Aufbau der Arbeit

In dieser narrativen Arbeit wurde die neueste Literatur auf dem Gebiet der Sportimmunologie in Zusammenhang mit dem mitochondrialen Immunstoffwechsel zusammengetragen. Die Forschungsfrage lautete: „Was ist über die Effekte von akuter Belastung, langfristigem Training und der kardiorespiratorischen Fitness (CRF) auf den mitochondrialen Immunzellmetabolismus bekannt?

Verwendete Literatur

Die Literaturrecherche wurde in der Datenbank PubMed durchgeführt. Eingeschlossen wurden Studien, welche die Oxygraph- oder Seahorse-Technik zur Messung der mitochondrialen Funktion in verschiedenen Immunzellen im Zusammenhang mit Sport bei gesunden und erkrankten Personen verwendeten.

Ergebnisse und Diskussion

Es wurden 20 Studien eingeschlossen. Dabei wurden PBMCs (peripheral blood mononuclear cells), Lymphozyten, T-Zellen, NK-Zellen, Thrombozyten und Neutrophile untersucht. Während die Daten zur mitochondrialen Funktion durch akute sportliche Belastung widersprüchliche Ergebnisse zeigten, berichteten die Trainingsinterventionsstudien eine höhere mitochondriale Funktion in Immunzellen durch regelmäßige körperliche Aktivität. Auch zeigten sich Korrelationen zur CRF. Umgekehrt schien die mitochondriale Funktion bei verschiedenen Patientengruppen im Vergleich zu gesunden Kontrollgruppen eingeschränkt zu sein. Dabei wurden ebenfalls Korrelationen zum Schweregrad der Erkrankungen und verschiedenen Risikofaktoren gefunden.

Was ist neu und relevant?

Die CRF und damit verbunden die Funktion der Mitochondrien spielen eine essentielle Rolle im Kontext von Gesundheit und Krankheit. Auch für Immunzellen ist ein effektiver Stoffwechsel von entscheidender Bedeutung für den Ablauf zentraler zellulärer Funktion. Wenn ein regelmäßiges Ausdauertraining mit einer Verbesserung der mitochondrialen Atmung der Immunzellen einhergeht, könnte sportliche Aktivität im Rahmen der Optimierung der Immunfunktion in Prävention und Therapie eine zusätzliche bedeutsame Rolle zukommen.

Methodische Einschränkungen

1. Heterogene Immunzellpopulationen: Ein Großteil der Studien befasste sich mit PBMCs, welche sich aus mehreren Immunzellen zusammensetzen.
2. Heterogene Studienpopulationen: Die Studien umfassten verschiedene Studienkollektive mit Sportlern, Patienten oder Inaktiven.
3. Heterogene Belastungsprotokolle: Unterschiedlich intensive Belastungen könnten für die widersprüchlichen Ergebnisse der Akutbelastung sorgen.
4. Narrativer Review: Um einen Überblick über das Thema zu geben, wurde ein systematisches Vorgehen vernachlässigt.

Schlussfolgerungen für die Praxis

- Ein langfristiges regelmäßiges Ausdauertraining mit Steigerung der $\dot{V}O_2$peak ist mit einer Erhöhung der maximalen Atmungsfähigkeit verschiedener Immunzellen assoziiert.
- Sowohl die CRF als auch die mitochondriale Funktion der Immunzellen könnten in der klinischen Praxis wichtige Kriterien zur Beurteilung des Gesundheitszustandes darstellen.
- Um einen kausalen Zusammenhang zu bestätigen, sind weitere gut kontrollierte Studien notwendig. ■

Article incorporates the Creative Commons Attribution – Non Commercial License.
https://creativecommons.org/licenses/by-nc-sa/4.0/

QR-Code scannen und Artikel online lesen.

KORRESPONDENZADRESSE:
Prof. Dr. Karsten Krüger
Justus Liebig University Giessen,
Department of Exercise Physiology and Sports Therapy, Institute of Sports Science
Kugelberg 62, 35394 Giessen, Germany
✉: Karsten.krueger@sport.uni-giessen.de

Supplement

SUPPLEMENT

Gebhardt K, Krüger K. Effects of cardiorespiratory fitness on immune cell mitochondrial metabolism in health and disease. Dtsch Z Sportmed. 2024; 75: 123-131. doi:10.5960/dzsm.2024.587

Table S1 – Part 1

Study interventions. IG=Intervention group; HC=Healthy controls; CG=Control group; HIIT=High-Intensity-Interval-Training; MICT=Moderate-intensity training; PAH=Pulmonary artery hypertension.

AUTHOR, YEAR	METHOD	CELL TYPE	STUDY POPULATION	PARTICIPANTS (N)	AGE	$\dot{V}O_2$PEAK AT BASELINE (ML/KG/MIN)	EXERCISE PROGRAM	DURATION	FREQUENCY, INTENSITY, VOLUME PER SESSION	CONTROL/ HEALTHY GROUP
Janssen et al. (2022)	Seahorse	PBMCs	Healthy; Young females adults	N: 31 High fit: 15 Low fit: 16	High fit: median: 21.8 (21.6-23.7) Low fit: Median: 24.0 (21.3-25.5)	High fit: 50.4 (49.0-54.0) Low fit: 35.1 (32.2-35.7)	Single bout of exercise	-	60 min, 70% $\dot{V}O_2$peak	High-fit≥47 Low-fit≤37
Liepinsh et al. (2020)	Oxygraph	PBMCs	Healthy sedentary adults	N: 12	Mean: 36.2±7.3	Mean: 33.3±1.3	low-intensity cycling exercise	-	60 min, 50 W	-
Stampley et al. (2023)	Oxygraph	PBMCs and subtypes	Collegiate swimmers	N: 11	Median: 20 (IQ: 20, 20)	-	Maximal exercise swimming bout	-	Until exhaustion	-
Theall et al. (2021)	Oxygraph	PBMCs	Healthy active adults	N: 21	Mean: 27.0 ±5.4	Mean: 36.5±6.3	Single bout of exercise	-	30 min at 65-75% $\dot{V}O_2$peak	-
Hedges et al. (2019)	Oxygraph	PBMCs	Young healthy men	N: 10	Mean: 24.7 ±1.3	-	HIIT	2 weeks	3 days/ week, 8-12 intervals with 60s Wpeak with 75s 30W	-
Kocher et al. (2017)	Seahorse	PBMCs	Sedentary HIV+ patients	N: 7	Range: 36 - 58 years	-	Light Aerobic exercise	12 weeks	3 days/ week, 20-40 min, 50-80% HRR	-
Noz et al. (2019)	Oxygraph	PBMCs	Participants with increased cardiovascular risk	N: 16	Mean: 64±6	-	Low-intensity physical activity	16 weeks	At least 2 minutes after 30minutes sedentary behavior	-
Brand et al. (2020)	Oxygraph	Lymphocytes	Occupational burnout	N: 24 IG: 12 HC: 12	IG: Mean: 45.8±6.8 HC: Mean: 45.65±6.86	-	intervention involving physical activity	12 weeks	3 days/ week, 60 min, 60-75% HF_{max}	HC without burnout
Tsai et al. (2016)	Oxygraph	Lymphocytes	Healthy sedentary males	N: 60 HIIT: 20 MICT: 20 CG: 20	HIIT: Mean: 23.0±1.7 MICT: Mean: 22.1±0.9 CG: Mean: 22.5±1.3	HIIT: Mean: 34.0±1.4 MICT: Mean: 33.1±1.2 CG: Mean: 32.2±1.0	Cycling HIIT or MICT under hypoxic stress	6 weeks	5 days/ week, 30 min/ day HIIT: 5 intervals with 3 min 80% and 3 min 40% of $\dot{V}O_2$peak MICT: 30 min at 60% $\dot{V}O_2$peak	CG without exercise
Andonian et al. (2022)	Seahorse	CD4+ T cells	Rheumatoid arthritis	N: 12	Mean: 63.9±7.2	Mean: 24.9±6.6	HIIT	10 weeks	3 days/ week, 10 intervals with 60-90s with 80-90% and 50-60% HRR	-
Lin et al. (2022)	Oxygraph	NK cells	Sedentary young males	N: 60 HIIT: 20 MICT: 20 CG: 20	HIIT: Mean: 22.2±2.1 MICT: Mean: 22.3±2.8 CG: Mean: 22.6±2.7	HIIT: Mean: 33.5±4.8 MICT: Mean: 33.2±4.5 CG: Mean: 32.2±4.1	HIIT or MICT graded exercise test before and after interventions	6 weeks	5 days/ week HIIT: 5 intervals with 3min 80% and 3 min 40% of $\dot{V}O_2$peak MICT: 30 min at 60% $\dot{V}O_2$peak	CG without exercise
Hsu et al. (2019)	Oxygraph	Platelets	Stroke patients	N: 30 IG: 15 CG: 15	IG: Mean: 55.7±3.0 CG: Mean: 57.8±3.9	mL/min: IG: Mean: 11.55± 8.0 CG: Mean: 11.65±7.6	Cycling + general rehabilitation	4 weeks	5 days/ week, 30 min at 60% $\dot{V}O_2$peak	CG: general rehabilitation
Lin et al. (2021)	Oxygraph	Platelets	Peripheral artery disease	IG: 20 CG: 20	IG: Mean: 71.1±1.5 CG: Mean: 70.5± 1.9	mL/min: IG: Mean: 12.3±0.6 CG: Mean: 12.4±0.4	Cycling + general rehabilitation	12 weeks	3 days/ week, 30 min at ventilator threshold (VT)	CG: General rehabilitation

Gebhardt K, Krüger K. Effects of cardiorespiratory fitness on immune cell mitochondrial metabolism in health and disease. Dtsch Z Sportmed. 2024; 75: 123-131. doi:10.5960/dzsm.2024.587

SUPPLEMENT

Table S1 – Part 2

Study interventions. IG=Intervention group; HC=Healthy controls; CG=Control group; HIIT=High-Intensity-Interval-Training; MICT=Moderate-intensity training; PAH=Pulmonary artery hypertension.

AUTHOR, YEAR	METHOD	CELL TYPE	STUDY POPULATION	PARTICIPANTS (N)	AGE	VO_2PEAK AT BASELINE (ML/KG/MIN)	EXERCISE PROGRAM	DURATION	FREQUENCY, INTENSITY, VOLUME PER SESSION	CONTROL/ HEALTHY GROUP
Bartlett et al. (2020)	Seahorse	Neutrophils	Prediabetic overweight-obese older adults	N: 16 IG: 10 HC: 6	IG: Mean: 71±5 HC: Mean: 23±1	-	low-volume high-intensity interval walking	10 weeks	3 days/ week, 30 min, 10 intervals with 60s 50-60% HRR and 80-90% HRR	HC: healthy young adults
Li et al. (2015)	Oxygraph	PBMCs	Early-stage heart failure patients	N: 49 IG: 25 HC: 24	IG: Mean: 49±3 HC: Mean: 47±3	-	-	-	-	HC
Shirakawa et al. (2019)	Oxygraph	PBMCs	Mild CHF (NYHA class I-II) or moderate to severe CHF (NYHA class III)	N: 62 NYHA class I-II: 31 NYHA class III: 31	NYHA class I-II: Mean: 63±13 NYHA class III: Mean: 61±14	NYHA class I-II: Mean: 19.5±4.6 NYHA class III: Mean: 14.5±4.3	-	-	-	-
Sommer et al. (2022)	Oxygraph	PBMCs	Pulmonary Arterial Hypertension	N: 39 In patient PAH: 14 Outpatient PAH: 15 CG: 10	Inpatient: Median: 59 (50; 70) Outpatient: 59 (49; 64) CG: 56 (28;68)	-	-	-	-	CG
Tyrrell et al. (2015)	Seahorse	PBMCs	Overweight, obese older adults	N: 15	Mean: 68.3±3.5	-	-	-	-	-
Zhou et al. (2020)	Oxygraph	PBMCs	Heart failure hospitalized patients	N: IG: 19 HC: 19	IG: Mean: 51.8±14.1 HC: Mean: 42.3±8.0	-	-	-	-	HC
Gamradt et al. (2021)	Seahorse	T cells	Major depressive disorder	N: 56 IG: 28 HC: 28	IG: Mean: 32.2±11.7 HC: Mean: 32.2±10.5	-	-	-	-	CG

SUPPLEMENT

Gebhardt K, Krüger K. Effects of cardiorespiratory fitness on immune cell mitochondrial metabolism in health and disease. Dtsch Z Sportmed. 2024; 75: 123-131. doi:10.5960/dzsm.2024.587

Table S2 – Part 1

Overview and summary of the findings across the included studies. OXPHOS=Oxidative phosphorylation; C=Correlation.

AUTHOR, YEAR	CELL TYPE	VARIABLES	ACUTE	TRAINING	IG VS. CG	CORRELATION
Janssen et al. (2022)	PBMCs	PBMC number	↓	-	↔	-
		PBMC composition	↔	-	↔	-
		Basal respiration	↔	-	↑	-
		Maximal respiration	↔	-	↑	-
		Mitochondrial respiratory capacity	↔	-	↑	-
		Spare respiratory capacity	↔	-	↑	-
		ATP linked respiration	↔	-	↑	-
		Proton leak	↔	-	↑	-
Liepinsh et al. (2020)	PBMCs	Basal respiration	↑	-	-	-
		FA-dependent leak	↑	-	-	-
		OXPHOS	↑	-	-	-
		FA-dependent OXPHOS coupling efficiency	↑	-	-	-
		Complex-IV-linked respiration at OXPHOS state	↔	-	-	-
Stampley et al. (2023)	PBMCs	PBMC number	↑	-	-	-
		Basal respiration	↑	-	-	-
		Other states	↔	-	-	-
		Flux control ratio	↑	-	-	-
Theall et al. (2021)	PBMCs	Total mitochondrial respiration	↔	-	-	-
Hedges et al. (2019)	PBMCs	$\dot{V}O_2$peak	-	↑	-	-
		Total mitochondrial respiration	-	↔	-	-
Kocher et al. (2017)	PBMCs	$\dot{V}O_2$peak	-	↑	-	No C. between $\dot{V}O_2$peak and mitochondrial function.
		Total mitochondrial respiration	-	↑	-	
		Basal respiration	-	↔	-	
		Respiratory capacity	-	↑	-	
		Spare respiratory capacity	-	↑	-	
		Non-mitochondrial respiration	-	↑	-	
Noz et al. (2019)	PBMCs	Basal respiration	-	↔	-	-
		Maximal respiration	-	↓	-	
Brand et al. (2020)	Lymphocytes	Total mitochondrial respiration	-	↑	↓	Positive C. between symptoms of depression and burnout with mitochondrial activity.
		OXPHOS	-	↑	↓	
		Maximal respiration	-	↑	↓	
		Symptoms of depression and burnout	-	↓	↓	
Tsai et al. (2016)	Lymphocytes	$\dot{V}O_2$peak	-	↑	↑	-
		Total mitochondrial respiration	-	↑	↑	
		OXPHOS	-	↑	↑	
		ATP linked respiration	↓	↑	↑	
		Reserve capacity	↓	↑	↑	
Andonian et al. (2022)	CD4+ T cells	$\dot{V}O_2$peak	-	↑	-	Positive C. between CRF and changes in basal and maximal respiration.
		Total mitochondrial respiration	-	↔	-	
Lin et al. (2022)	NK cells	$\dot{V}O_2$peak	-	-	-	Positive C. between changes of $\dot{V}O_2$peak and changes in reserve and maximal respiration.
		Maximal respiration	↑	↑	-	
		Reserve oxygen capacity	↑	↑	-	

Gebhardt K, Krüger K. Effects of cardiorespiratory fitness on immune cell mitochondrial metabolism in health and disease. Dtsch Z Sportmed. 2024; 75: 123-131. doi:10.5960/dzsm.2024.587

SUPPLEMENT

Table S2 – Part 2

Overview and summary of the findings across the included studies. OXPHOS=Oxidative phosphorylation; C=Correlation.

AUTHOR, YEAR	CELL TYPE	VARIABLES	ACUTE	TRAINING	IG VS. CG	CORRELATION
Hsu et al. (2019)	Platelets	$\dot{V}O_2$peak	-	↑	↑	Positive C. between changes of $\dot{V}O_2$peak and changes in OXPHOS and maximal respiration.
		Routine respiration	-	↔	↔	
		OXPHOS	-	↑	↑	
		Maximal respiration	-	↑	↑	
		Oxidative stress	-	↓	↓	
		Pro-inflammatory status	-	↓	↓	
Lin et al. (2021)	Platelets	$\dot{V}O_2$peak	-	↑	↑	Positive C. between changes of $\dot{V}O_2$peak and changes of OXPHOS and maximal respiration.
		Maximal respiration	-	↑	↑	
		OXPHOS	-	↑	↑	
		Oxidative stress	-	↓	↓	
		Pro-inflammatory status	-	↓	↓	
Bartlett et al. (2020)	Neutrophils	Basal respiration	-	↑	–	Negative C. between relative $\dot{V}O_2$peak and changes in HbA1c and fasting glucose.
		Maximal respiration	-	↑	–	Positive C. between relative $\dot{V}O_2$peak and fasting insulin.
		ATP production	-	↑	–	
		Proton leak	-	↓	–	
Li et al. (2015)	PBMCs	Total mitochondrial respiration	-	-	↓	Negative C. between mitochondrial respiratory function and oxidative stress and inflammation.
		Basal respiration	-	-	↓	Negative C. between mitochondrial respiratory function and TNF-α, IL-6, CRP, SOD.
		Maximal respiration	-	-	↓	Negative C. between mitochondrial respiratory function and cardiometabolic risk factors.
		Anti-oxidative capacity	-	-	↓	
Shirakawa et al. (2019)	PBMCs	$\dot{V}O_2$peak	-	-	↓	Negative C. between $\dot{V}O_2$peak and mitochondrial ROS levels.
		Mitochondrial respiratory capacity	-	-	↓	
		Maximal respiration	-	-	↓	
		Mitochondrial ROS generation	-	-	↑	
Sommer et al. (2022)	PBMCs	Mitochondrial respiration	-	-	↔	Negative C. between mitochondrial respiration and disease severity.
Tyrell et al. (2015)	PBMCs	-	-	-	–	Positive C. between spare respiratory capacity and maximal respiration and knee extensor strength.
						Negative C. between basal respiration, maximal respiration, spare respiratory capacity and IL-6.
Zhou et al. (2020)	PBMCs	Maximal respiration	-	-	↓	-
		Pro-inflammatory gene expression	-	-	↑	
Gamradt et al. (2021)	T cells	Total mitochondrial respiration	-	-	↓	Negative C. between basal respiration, ATP-linked respiration, spare respiratory capacity and clinical-rated depression severity.
		Basal respiration	-	-	↓	
		ATP production	-	-	↓	
		Spare respiratory capacity	-	-	↓	