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# 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

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## 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 ›



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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) | $VO_2$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)<br>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<br>MICT: Mean: 33.1 ± 1.2<br>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<br>MICT: Mean: 33.2 ± 4.5<br>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:<br>IG: Mean: 11.55 ± 8.0<br>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<br>NYHA class I-II: 31<br>NYHA class III: 31 | NYHA class I-II: Mean: 19.5 ± 4.6<br>NYHA class III: Mean: 14.5 ± 4.3 | - |
| **Sommer et al. (2022)** | PBMCs | Pulmonary Arterial Hypertension | N: 39<br>In patient PAH: 14<br>Outpatient PAH: 15<br>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.

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