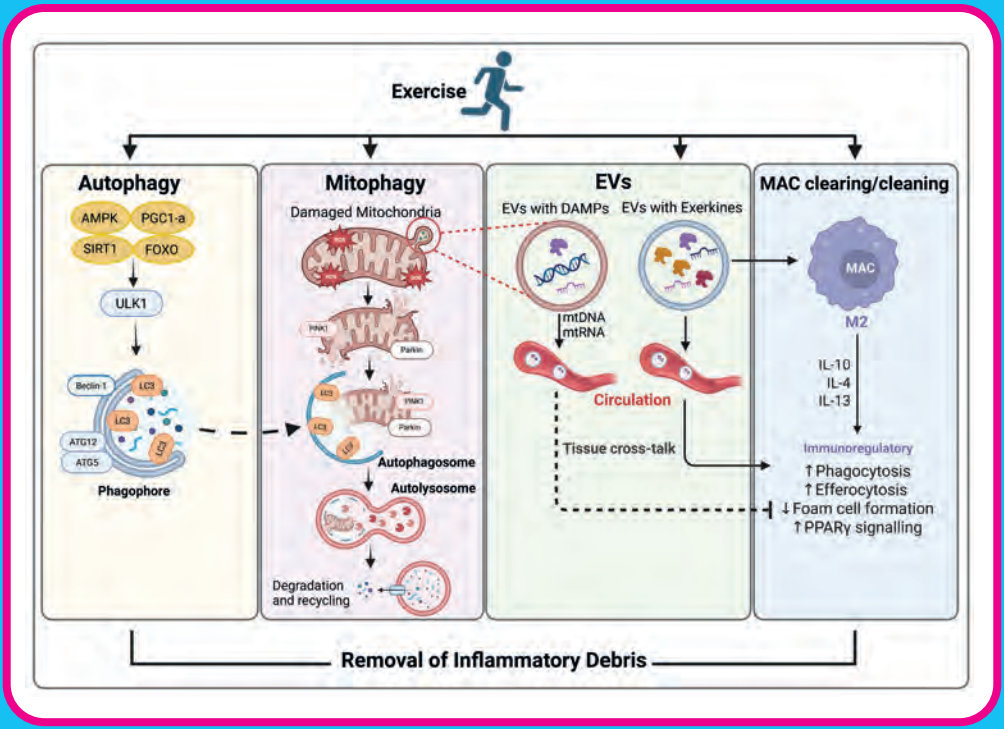
EXERCISE IMMUNOLOGY REVIEW



VOLUME 31 • 2025

The International Society of
Exercise and Immunology

EXERCISE IMMUNOLOGY REVIEW

An official Publication of the
International Society of Exercise and
Immunology (ISEI)

EXERCISE IMMUNOLOGY REVIEW

VOLUME 31 · 2025

Contents

Exercise Immunology Review

Editorial Statement

Exercise Immunology Review, an official publication of the International Society of Exercise Immunology and of the German Society of Sports Medicine and Prevention, is committed to developing and. enriching knowledge in all aspects of immunology that relate to sport, exercise, and regular physical ativity. In recognition of the broad range of disciplines that contribute to the understanding of immune function, the journal has adopted an interdisciplinary focus. This allows dissemination of research findings from such disciplines as exercise science, medicine, immunology, physiology, behavioral science, endocrinology, pharmacology, and psychology.

Exercise Immunology Review publishes review articles that explore: (a) fundamental aspects of immune function and regulation during exercise; (b) interactions of exercise and immunology in the optimization of health and protection against acute infections: (c) deterioration of immune function resulting from competitive stress and overtraining; (d) prevention or modulation of the effects of aging or disease (including HIV infection; cancer; autoimmune, metabolic or transplantation associated disorders) through exercise. (e) instrumental use of exercise or related stress models for basic or applied research in any field of physiology, pathophysiology or medicine with relations to immune function.

Exercise Immunology Review (ISSN 1077-5552)

Postmaster:
Prof. Dr. Karsten Krüger
Department of Exercise Physiology and Sports Therapy
Institute of Sports Science, University of Giessen, Germany
Kugelberg 62 | 35394 Giessen

Send editorial correspondence to:
Karsten Krüger
karsten.krueger@sport.uni-giessen.de

to subscribe or renew subscription go to https://exerciseimmunology.com/

Occurrence of acute respiratory illnesses in athletes: a systematic review and meta-analysis

Wilma Grönroos[a,*], Matti Uhari[b], and Olli Ruuskanen[c]

[a] Paavo Nurmi Centre and Unit for Health and Physical Activity, University of Turku, Turku 20520, Finland
[b] PEDEGO Research Unit, University of Oulu and Department of Pediatrics and Adolescence, Oulu University Hospital, Oulu 90029, Finland
[3] Department of Pediatrics and Adolescent Medicine, Turku University Hospital and University of Turku, Turku 20521, Finland

ABSTRACT

Background: Estimating the occurrence of acute respiratory illness (ARI) in athletes is crucial for understanding the need for preventive measures. This study aimed to estimate the occurrence of ARIs in athletes compared to non-athlete controls. We evaluated which of the published studies on the occurrence of ARI in athletes were eligible, giving due consideration to the epidemic nature of viral ARIs.

Methods: We performed a systematic search of PubMed, EBSCOhost, and Web of Science databases from January 1990 to May 2023. Only studies reporting the occurrence of ARIs in athletes, with a duration of at least 12 months and simultaneously employing non-athlete controls, were included. The random effects model was used to calculate the incidence rate ratio (IRR) of ARI in athletes compared to non-athlete controls, with 95% confidence intervals.

Results: Our search yielded 218 results, of which 6 studies met the essential criteria for viral ARIs and were included in our meta-analysis. Since the exact number of ARIs was not reported in many studies, we had to estimate the total number of ARIs for both athletes and non-athlete controls. The occurrence of ARIs was 1.87 times higher in athletes compared to non-athlete controls (3.2 vs 1.7, pooled IRR 1.87, 95% CI 1.08 to 3.26). Publication bias analysis or a funnel plot was not evaluated because the primary objective of none of the studies was to determine the occurrence of ARI in athletes. Most of the studies on the occurrence of ARIs in athletes had a duration of less than 12 months and did not include a concurrent control group, making them ineligible considering the seasonal and contagious nature of ARIs.

Conclusion: Our meta-analysis suggests that athletes suffer from significantly more ARIs than non-athletic subjects. Our observations highlighted the lack of high-quality long-term studies on the occurrence of ARIs in athletes.

**Corresponding author:*
Wilma Grönroos, MD,
Paavo Nurmi Centre and Unit for Health and Physical Activity,
University of Turku, Turku 20520, Finland, Tel: +358 50 4053252, email address: wgegro@utu.fi

Declarations

Funding:	Jenny and Antti Wihuri Foundation
Competing interests:	The authors declare that they have no competing interests.
Ethics Approval:	Not applicable.
Consent to Participate:	Not applicable.
Consent for Publication:	Not applicable.
Availability of Data and Material:	All data generated or analyzed during this study are included in this published article.
Code Availability:	Not applicable.
Authors' Contributions:	All authors drafted and edited the manuscript, read and approved the final version of it and agree with the order of presentation of the authors.

ORCIDs:
Wilma Grönroos: 0000-0001-9488-6946
Olli Ruuskanen: 0000-0002-4354-1187
Matti Uhari: 0000-0003-2965-8466

INTRODUCTION

Acute respiratory illnesses (ARIs) are the most common complaints in athletes, especially during major sports events, accounting for up to 50% of all medical consultations (23). In clinical practice, ARIs are usually classified as viral upper respiratory tract infections ("common cold") (136, 138). At the Olympics and World Championships, an ARI may ruin the long-term goal of an athlete. The occurrence and the disease burden are, however, both controversial (14, 113). In many studies, the occurrence during major sports events has been 2-5 % (2, 37, 119, 120), which is consistent with or even lower than in the general population (7, 129). In contrast, some recent studies have reported a physician-verified ARI of 38-45 % in athletes during major winter sports events (136, 138). The reasons for this disagreement are not well explained. Laboratory identification of the virus is the gold standard for verification of ARIs, but this is rarely performed in studies of athletes (19, 123, 138). Post the COVID epidemic, the increasing use of rapid diagnostics for point-of-care testing may address this lack of information, at least for common respiratory viruses.

Understanding the occurrence of ARIs in athletes is crucial for determining the proper prevention measures for reducing the burden of illness as well as for non-pharmaceutical interventions to minimize the contract and transmission of viral ARIs within sports teams (138). A recent systematic review and meta-analysis by a subgroup of the International Olympic Committee (IOC) consensus reported an annual ARI incidence of 1.7 in athletes (23). The IOC review reported a lower incidence of ARIs in elite athletes compared to non-elite athletes (23). These rates are comparable to, or even lower than, those in the general population. In 3 studies of young adults, the annual occurrence of ARI ranged between 2.3 and 4.6 (7, 13, 69).

To determine the incidence rate ratio of ARI in athletes compared to non-athlete controls, we performed a meta-analysis of the strictly selected studies published between January 1990 and May 2023.

METHODS AND SELECTION RATIONALE

Requirements for considering studies for meta-analyses of viral ARIs

The duration of the study should be at least 12 months to cover all annual outbreaks of the numerous respiratory viruses (*Table 1*). Most respiratory viruses annually display marked seasonal variation. For example, influenza epidemics occur in the Northern Hemisphere during the winter months and in the Southern Hemisphere during the summer months of the Northern Hemisphere (6, 86). The epidemics last 2-4 months and between epidemics the occurrence falls to near zero. The most common respiratory virus, rhinovirus, can be detected throughout the year, but distinct peaks occur in early autumn and late spring (43, 67, 75, 104). Finally, in some countries like Finland, respiratory syncytial virus (RSV) epidemics occur only every other year in 2-year cycles (108, 143). Therefore, if the study is conducted outside or during an epidemic, the study could result in either an underestimation or an overestimation of the occurrence of ARIs. The timing of epidemics may vary by weeks even in close geographical areas. Thus, athletes and controls may seem to have contracted a different number of ARIs in a study with only a short follow-up time.

A concurrent control group is needed when examining the occurrence of ARI in specified groups. The incidence of respiratory viruses varies from year to year. For example, the seasonal incidence of influenza is often approximated to be 5%-20% (132). Non-athlete controls should be matched for age and sex, and their weekly exercise should be known. In elite athletes, the mean training volume varies from 15-20 hours per week compared to less than 6 hours in the normal population (70, 107).

The prospective nature of the study is essential. It is difficult for anyone to reliably remember how many infections they have had even during the previous month. Athletes and controls might recall these differently, causing a recall bias in the results.

In clinical practice, ARI episodes among athletes are usually classified as common colds i.e., mild, self-limiting, viral upper respiratory tract infections (113, 136, 138). Symptoms typically include an acute onset of a sore throat, sneezing, a runny nose, nasal congestion, and a cough. Fever is usually absent or mild (60, 77). Flu-like illnesses with fever, muscle aches, and fatigue are rare (77). It is recommended that questions regarding all these symptoms be included in a standardized symptoms diary. Reminder emails for missing reports are essential. Web-based monitoring of study subjects has rarely been implemented (7, 49). In a study utilizing internet-based syndrome monitoring for ARIs in the general population of Germany, the participants received a weekly questionnaire via email to assess whether they had experienced an ARI in the past week (7).

Respiratory viral infections may occur as asymptomatic, paucisymptomatic, or symptomatic. A laboratory-based study in which 502 university students were followed for 8 weeks (September-October) found that the incidence rate of human rhinovirus was 8.3% per week for asymptomatic students (53). In a multicenter prospective cohort study of healthcare workers, the percentage of asymptomatic and paucisymptomatic influenza A cases was 47% and 42%, respectively (9). Similar observations were made in a community-based study, where 13% and 11% of 235 virologically confirmed cases of influenza virus infections were paucisymptomatic and asymptomatic, respectively (66). A study on 398 aquatic elite athletes, who had had SARS-CoV-2 infection, found that 54% had had a mild infection and 17 % reported no symptoms (70). One study found that having only 1 symptom for 1 day can prove to be a virus-positive ARI (136). In the occurrence studies on athletes, commonly used symptom algorithms, such as the Wisconsin Upper Respiratory Symptoms Survey (WURSS) or the Jackson Cold Scale, both require at least 2 symptoms that last for at least 2 days to diagnose an ARI; consequently, this may inadvertently exclude a marked percentage of ARIs. These algorithms may no longer be fit for purpose in assessing ARIs

in athletes. The Jackson Cold Scale was established in 1958 and the WURSS was not originally planned for diagnostic purposes but for a health-related quality of life questionnaire. Neither of these algorithms has been virologically validated. The World Health Organization (WHO) defines ARI as the sudden onset of symptoms of a sore throat and/or runny nose, and/or cough, with at least one of these symptoms being present (146).

ARI can be diagnosed clinically. Viral diagnostics can be used to confirm a viral ARI. Commercial multiplex PCR tests covering 16-18 viruses, are simple, sensitive, and reliable and have also been used as point-of-care tests. The viral etiology of an ARI can be established in 70-90% of cases in athletes (89, 111, 136, 138). In contrast, 2 previous studies assessing the viral etiology of ARIs in elite athletes found confirmed ARI incidences to be only around 30% (19, 123). Different respiratory viruses cannot be distinguished by their clinical features alone (44, 76).

The motivation to report all symptoms should be the same among athletes and controls. Active and regular viral diagnostics can be used to verify this. Discrepancies between the number of viral findings and the reported symptoms between the compared groups could indicate that there is reporting bias.

Data sources and search strategy

The review and protocol were not registered but were conducted according to published Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (96). Three databases, comprising PubMed, EBSCOhost, and Web of Science, were searched for studies published between January 1, 1990, and May 31, 2023. The following search phrase was used: ("acute respiratory illness*" OR "respiratory infection*" OR "respiratory tract infection*" OR "common cold*") AND (athlet* OR sport* OR exercis*) AND (prevalence* OR incidence* OR occurrence*). References that were potentially eligible after screening the title and abstract were reviewed and evaluated for inclusion via the full text. Further, additional papers were identified based on the reference list of relevant papers including review articles. The 124 studies included in the meta-analysis of the IOC report (23) were reanalyzed separately, with due consideration given to the contagious and seasonal nature of ARIs.

Inclusion and exclusion criteria

Studies were included for meta-analysis if they fulfilled the following criteria: (1) the duration of the study was at least 1 year, and (2) the study simultaneously included non-athlete controls. Because of the low number of prospective studies, retrospective studies that met the above criteria were also included in the meta-analysis. Studies that did not present any original data (reviews, editorials, etc.) were excluded. Only English-written reports were included (Figure 1). A common quality assessment of the included articles was undertaken using the Newcastle-Ottawa scale (145). Disagreements between authors were resolved by regular group discussions.

Data extraction

The following data were extracted from each of the included studies: the number of participants in each group, the duration of the study, and the number of ARI cases. One author extracted the data while a second author cross-checked the collected data.

There was only one study that reported the exact number of ARIs in athletes and non-athletes (12), and one study that reported the monthly number of ARIs per individual (61). The number of ARIs was determined by multiplying the monthly mean number of ARIs by the number of individuals in the group. One study reported the percentage of athletes and controls with ARI at 8 time points (38). In this study, the number of ARIs was determined by summing the total number of individuals with ARIs at each point. Two studies reported that individuals experienced either ≤2 episodes, 3-4 episodes, or ≥5 episodes (134, 135). We determined that individuals had either 1.5 episodes, 3.5 episodes, or 6 episodes, respectively. A further study also reported the number of ARIs similarly, and therefore we determined that individuals who reported 1-2 episodes were classified as having 1.5 episodes and individuals who had 3 or more episodes were classified as having 4 episodes (16).

Statistical analysis

The studies were pooled together in the meta-analysis. A random-effects model was chosen because of the expected heterogeneity between the studies. An incidence rate ratio (IRR) with 95% confidence intervals (CIs) was calculated. A StatsDirect version 3.3.6 was used in all the statistical analyses (StatsDirect Ltd, Wirral, UK).

Table 1. Requirements for a study included in meta-analyses of the occurrence of acute respiratory infections in athletes (see section 2.1). ARI = acute respiratory illness.

1. ESSENTIAL REQUIREMENTS
Simultaneous non-athlete controls
Duration of the follow-up at least one year
2. OTHER ASPECTS TO BE CONSIDERED
Weekly (online) monitoring using standardized symptom diaries
Athlete's exercise types and durations are homogenous
Standardized definition of ARI
Viral diagnostics of illness episodes
Motivation to report all symptoms should be the same among athletes and controls

RESULTS

The analysis of the 6 studies in the meta-analysis based on the selection criteria of the study

Our database search identified 1399 records. These were supplemented with 95 articles identified via references in relevant articles. Altogether 218 studies reported the occurrence of ARI in athletes. From these 218 studies, 192 did not include simultaneous non-athlete controls and were therefore excluded. A further 18 studies were then excluded because the duration of the follow-up was less than 1 year. In total, 8 studies fulfilled the essential requirements. One study did not report the number of ARIs in the control subjects. One study was excluded because the population was already included in another study. Finally, 6 studies were included in the meta-analysis (Fig. 1, *Table 2*) (12, 16, 38, 61, 134, 135). The total number of athletes and non-athletes included in the meta-analysis was 388 and 344, respectively. Six different sports were covered: track and field (number of subjects, n=199) (16), football (n=75) (38), swimming (n=40) (134), wrestling (n=40) (135), cross-country skiing (n=19) (61), and triathlon (n=15) (12). Determining the occurrence of ARI in athletes was not the primary objective of any of the studies but rather, for example, the aim was to evaluate secretory immunoglobulin A or to determine vitamin D levels (38, 134, 135). Therefore, a publication bias analysis and a funnel plot were not evaluated.

In 4 of 6 studies included in the meta-analysis, the occurrence of ARI was significantly higher in athletes than in the non-athlete controls (Fig. 2) (38, 61, 134, 135). In the meta-analysis, the combined IRR (athletes vs. controls) was 1.87 (CI: 1.08-3.26). The annual frequency of ARI episodes was 3.2 in athletes and 1.7 in the non-athlete controls. Three studies were prospective (12, 38, 61), and the other three were retrospective (*Table 2*) (16, 134, 135). In 2 prospective studies, participants were monitored either daily or weekly using a symptom diary or log (12, 38). In 1 prospective study, two-thirds of the cases were verified by a physician, and the remainder were based on self-reported symptoms by the subjects (61). None of the studies defined ARI as paucisymptomatic (1 symptom only) or lasting only 1 day. In addition, none of the studies identified the etiology of the ARI.

The additional assessments of other supplemental studies

Four studies out of the 218 studies originally analyzed, but none in the meta-analysis used etiological diagnostics (3, 54, 123, 138). One study did not investigate the viral etiology of ARI; instead, it focused on an outbreak of group A Streptococcal pharyngitis among university students in a judo club (3). In another study, where symptomatic athletes were screened only for influenza during the 2002 Winter Olympiad, influenza A/B was diagnosed in 13 of 46 athletes (54). Two other studies investigated multiple respiratory pathogens. In one study of elite summer sport athletes conducted in Australia, infectious agents were identified in only 29% of the ARI episodes (123). In contrast, another study of elite winter sport athletes confirmed the viral etiology of ARI in 75% of the cases (138).

In a reanalysis of the IOC meta-analysis, we found that 4 out of the 124 studies included were eligible when considering the seasonal and contagious nature of ARIs (12, 16, 23, 38, 135). Five studies lasted for 1 year or more (12, 16, 38, 62, 135). The duration of 80 of the studies was 3 months or less (1-5, 10, 11, 20, 22-28, 30-37, 39, 41, 42, 44, 46-48, 51, 52, 54-59, 64, 65, 71, 72, 76, 79, 80, 83, 84, 87, 88, 90-94, 97, 99, 100, 102, 103, 105, 106, 109, 110, 113, 114, 116, 119, 120, 122, 124, 125, 127, 130, 131, 133, 139, 140, 144, 147, 148). Ten studies included a concurrent control group (12, 16, 38-41, 106, 109, 123, 135). One study also defined ARI as paucisymptomatic (138).

Due to the low number of controlled studies, we analyzed 8 uncontrolled studies but with a duration of 12 months or longer (*Table 3*). In 343 athletes the mean number of ARIs per person per year was 2.8 (range 0.2–8.4) (8, 17, 21, 29, 63, 68, 128, 149).

DISCUSSION

The occurrence of ARIs in athletes

We found 6 studies on the occurrence of ARIs in athletes that were controlled and lasted for 12 months or longer. The annual mean number of ARI episodes in athletes was 3.2, compared to 1.7 in the control subjects. In 8 uncontrolled studies with a duration of 12 months or more, the mean number of ARI episodes was 2.8 per year (*Table 3*). Our meta-analysis suggests that athletes suffer significantly more ARIs than non-athletic subjects (IRR 1.87). Our observations showed the lack of high-quality long-term studies on the occurrence of ARI in athletes. None of the studies included in the meta-analysis was specifically designed to evaluate ARI occurrence among athletes, and controlled studies on this topic remain limited.

Our observations differ from those of the IOC consensus report (23). Their technically well-executed analysis demonstrated a lower incidence of ARI among athletes, 4.7/1000 athlete days converted to approximately 1.7 ARIs per athlete per year compared to 3.2 in our analysis. The IOC report is the cornerstone analysis of ARIs in athletes but the studies included can be criticized for several reasons although the investigators considered 89% of the 124 studies excellent or good. Only 8 studies aimed to evaluate the occurrence of ARIs (72, 96, 106, 121, 123, 128, 138). The major limitation of the studies was the short duration not taking into account the seasonal and contagious nature and risk factors of viral ARIs. The studies carried out only during summer months may underestimate the occurrence of ARIs and studies carried out only during winter months may overestimate the occurrence. Furthermore, the varying admission criteria are subject to an error. The heterogeneity of disciplines is also a source of bias. Summer sports and team sports should be analyzed separately. In all studies, the inaccurate nature of self-reporting is an important weakness.

Our findings are consistent with the systematic review of Moreira et al., which reported on 30 studies published before 2009 (with 8575 athletes and 1789 non-athletes) (85). The

review found that athletes experience a higher rate of ARIs after training and competitions compared with less active individuals. Due to the high heterogeneity among the studies, Moreira and colleagues did not perform a meta-analysis. Hence, they refrained from providing numerical data regarding the occurrence of ARI among athletes and non-athletes (85).

The major limitation of our analysis is the low number and low quality of the studies included. Only 1 of the studies reported the exact number of ARIs in each group. For the remaining studies, we had to estimate the total number of ARIs in athletes and non-athletes. None of the studies defined ARI as paucisymptomatic or lasting only 1 day. Thus, a marked number of ARIs may have gone unrecorded. The retrospective nature of the 3 studies included in this meta-analysis might have hampered the number of ARIs. The meta-analysis included only 6 studies and had a small sample size (388 athletes and 344 non-athletes). Because of this, different sports disciplines, which may have differing effects on susceptibility to ARIs, could not be analyzed. The included studies covered only 6 different sports, and, for example, endurance athletes were not included. The primary objective of none of the studies was to determine the occurrence of ARI in athletes. For this reason, we did not report the publication bias analysis or the funnel plot. Furthermore, 95 studies were identified through sources other than database searching, suggesting that some studies may not have been identified.

Risk factors and effects of ARI

Vigorous and prolonged exercise temporarily suppresses the immune functions, and it has been believed to increase the risk of ARI in athletes (50, 73, 95, 115, 117), but its clinical meaningfulness has been questioned (14). A recently conducted systematic review found no evidence of detrimental effects from vigorous physical activity lasting up to 600 minutes per week (15). Many behavioral factors such as frequent use of public transportation and air travel, human crowding, shared housing and shared meals during training camps and competitions, full-contact sports, heavy breathing, and shouting by infected individuals during the game and in the locker room, are likely to increase the transmission of viral respiratory infections in athletes (77, 112); all of these being aspects that were ascertained as important during the COVID-19 pandemic. For example, Finnish elite athletes who competed in the 2019 Nordic World Ski Championships in Seefeld, Austria, had a 7-fold increase in the risk of ARI compared with control subjects exercising normally in Finland (136). During the 14-day study period, 38% of 26 athletes and 6% of 52 control subjects experienced symptoms of ARI. Compared with the support staff, who shared many risk factors with the athletes and of whom 17% of 36 reported ARI, athletes had a 2-fold risk of ARIs (136). Non-pharmaceutical interventions, such as the wearing of face masks, enhanced social distancing, and hand hygiene, can help to mitigate the contract and transmission of viral ARIs within sports teams (141). In addition, for athletes with recurrent infections, factors affecting immunity, such as diet and excessive psychological and physical stress, should be evaluated.

Psychological stress, sleep disturbance, fatigue, and nutritional deficiency may increase susceptibility to ARIs in athletes (14, 18, 101). A study investigating a cohort of elite young basketball players over 4 weeks during a competitive training period found a significant correlation between sources and symptoms of psychological stress and the total number of occurrences of ARI in the second week (84). During that week, participants had a greater source of psychological stress, and they reported a higher number of episodes of ARI (84). Similarly, another study of collegiate swimmers found that an increase in stress was associated with an increase in the ARI rate (142).

Age also affects susceptibility to respiratory viral infections. Adults aged 20-39 have higher rates of ARIs compared to the rate in adults over 40 (13, 82). Surprisingly, the IOC subgroup's meta-analysis, which included athletes up to 65 years old, found that the annual ARI incidence in athletes under 35 (5.9%) was about half that of athletes over 35 (9.2%) (23). Additionally, ARIs are more common in young adult women than in men of the same age which is likely to be related to greater exposure to young children (82). Indeed, children younger than 5 years of age in the same household are a major risk factor for virus transmission (13, 81, 82, 126). In a household-based surveillance study, the mean number of ARIs per person per year was 6.1 for children aged <5 years, and they were virus-positive for half of the year (13). Therefore, the number of children under 5 in an athlete's family should be known.

The type of sport and exercise may affect susceptibility to ARIs. Endurance exercise with a high cardiorespiratory component such as cross-country skiing, cycling, and running may cause a different susceptibility to infections than team sports and full-contact sports which involve close heavy breathing and shouting. For example, in one recent study on swimmers, open water swimmers were the least likely to acquire a SARS-CoV-2 infection, and water polo swimmers were most likely (70). Most open-water swimmers train on their own with less contact with others, and many train in ocean settings or saltwater pools, which may kill viruses. The use of common locker rooms increases the possibility of the transmission of viral infections. Instead of a high demand for endurance, some sports like climbing, gymnastics, and martial arts have a high resistance demand. In sports such as basketball, rugby, and swimming, on the other hand, endurance and resistance components are combined.

Laboratory confirmation of viral ARI is important. It has the potential for the identification and control of early outbreaks. An influenza epidemic may coincide with sports events, during which diagnosing influenza is crucial, as it can be treated and prevented with antivirals (54, 74). Additionally, an etiologic diagnosis is of importance for isolating infected athletes during competitions and training camps (77). Understanding the clinical characteristics and symptomatology of different respiratory viral infections in athletes can help address challenges related to the identification of ARIs. The occurrence and clinical significance of asymptomatic infections in athletes are unclear. There is only 1 study that has investigated the occurrence of asymptomatic respiratory viral infections in athletes. It reported that 8% of 26 athletes had an asymptomatic infection caused by the rhinovirus during a 2-week winter sports competition (136).

Table 2. Characteristics of studies included in the meta-analysis. ARI = acute respiratory illness.

Source	Country	Duration of study, d	Study desing	ARI definition	Symptom monitoring	Athletes				Controls				Controls	
						N	Sport	Mean (SD) age, y	Sex, No.	N	Sport	Mean (SD) age, y	Sex, No.	Athletes	Controls
Heir and Larsen, 1995	Norway	365	Prospective	2 or more local symptoms occurred for 2 or more days, or a single local symptom (excluding sneezing) lasted for 3 or more days	The subjects recorded all symptoms of respiratory infections, and two-thirds of the cases were verified by physicians.	19	Cross-country skiing	range 19-21	Male, 19	22	Military service recruits	Age matched	Male, 22	116	90
Chester et al, 2003	United Kingdom	365	Retrospective cohort study	colds, flu, sinusitis, coughs or sore throats	Retrospective questionnaire	199	Track and field	<20 yrs n=41 ≥20 <40 yrs n=140 ≥40 yrs n=18	Female, 72; male, 127	195	Largely university students	<20 yrs n=33 ≥20 <40 yrs n=163 ≥40 yrs n=6	Female, 128; male, 74	496,5	437,5
Fahlman and Engels, 2005	United States	365 (8 time points)	Prospective cohort	the infection lasted 3 or more days, and all 3 symptoms - cough, runny nose, and nasal congestion - were present througout the entire duration	A weekly log	75	Football	20.5 (1.5)	Male, 75	25	University students	20.5 (1.6)	Male, 25	174	19
Broadbent, 2011	Australia	365	Prospective cohort	Symptoms lasting for 3 days or more	A daily illness diary	15	Triathlon	30 (5)	N/A	12	Untrained men (no aerobic or resistance training or participation in recreational sports)	30 (6)	Male, 12	2	7
Umarov et al, 2018	Uzbekistan	365	Retrospective	N/A	N/A	40	Wrestling	range 19-24	Male, 40	60	healthy individuals of the same sex and age who were residents of the same country	Age matched	Male, 60	170	58,5
Umarov et al, 2019	Uzbekistan	365	Retrospective	Mild course: Presence of several symptoms of acute URTI, with no complications or symptom exacerbations	A self-reported questionnaire was completed in February and August	40	Swimmimg	range 19-24	Female, 40	30	healthy individuals of the same sex and age who were residents of the same country	Age matched	Female, 30	160	45,5

Table 3. Characteristics of uncontrolled studies with a duration of 12 months or longer. ARI = acute respiratory illness.

Source	Country	Duration of study	Study design	ARI/illness definition	Symptom monitoring	Athletes				No. of ARI	Mean number of ARIs per person per year
						N	Mean (SD) age, y	Sex, No.	Sport		
Clancy et al, 2006	Australia	12 months	Retrospective	N/A	Self-referral to a medical sports clinic due to fatigue, recurrent sore throats, and impaired performance	27	range 16.7-40.2	Female, 10; male, 17	N/A	63	2.3
Belli et al, 2010	Italy	29 months	Prospective	Any non-injury related medical condition requiring medication or treatment	N/A	35	37 (7)	Male, 35	Sailing	79	0.8
Hellard et al, 2011	France	24 months	Prospective	Signs or symptoms for >48 h, required medication, and missed at least one training session as a result of illness	Weekly by the two same physicians	18	range 19-30	Female, 8; male, 10	Swimming	109	3.7
Zehsaz et al, 2014	Iran	12 months	Retrospective	N/A	Questionnaire and interview	100	range 18-35	Male, 100	Predominantly endurance-based activities such as running, cycling, swimming, triathlon, and other sports	197	2.0
Svendsen et al, 2016	Norway	On average, 44 months per athlete	Retrospective cohort	One or more symptoms indicative of respiratory tract infection on two or more consecutive days, or when symptoms were severe enough that training was completely discontinued on at least 1 day	Symptoms were recorded in spreadsheet training diaries	37	18-34	Female, 17; male, 22	Cross-country skiing	410	3.0
Dumontier et al, 2020	Belgium	13 months	Prospective	For each symptom, the number of days was multiplied by the severity score (1-3) and then summed to provide the weekly upper respiratory tract symptoms score. A score of ≥ 12 was indicative of an upper respiratory tract symptoms episode	Weekly questionnaire	18	16.4 (3.4)	Female, 18	Gymnastics	33	1.7
Jeffries et al, 2020	Australia	12 months	Prospective	Upper respiratory signs and symptoms for ≥48 hours	the Wisconsin Upper Respiratory Tract Infection Survey	16	26.1 (5.1)	Female, 9; male, 7	Contemporary dance	134	8.4
Crunkhorn et al, 2022	Australia	12 months	Prospective	A complaint or disorder not related to injury	By doctors and physiotherapists	92	N/A	Female, 34; male, 58	Sailing	22	0.2

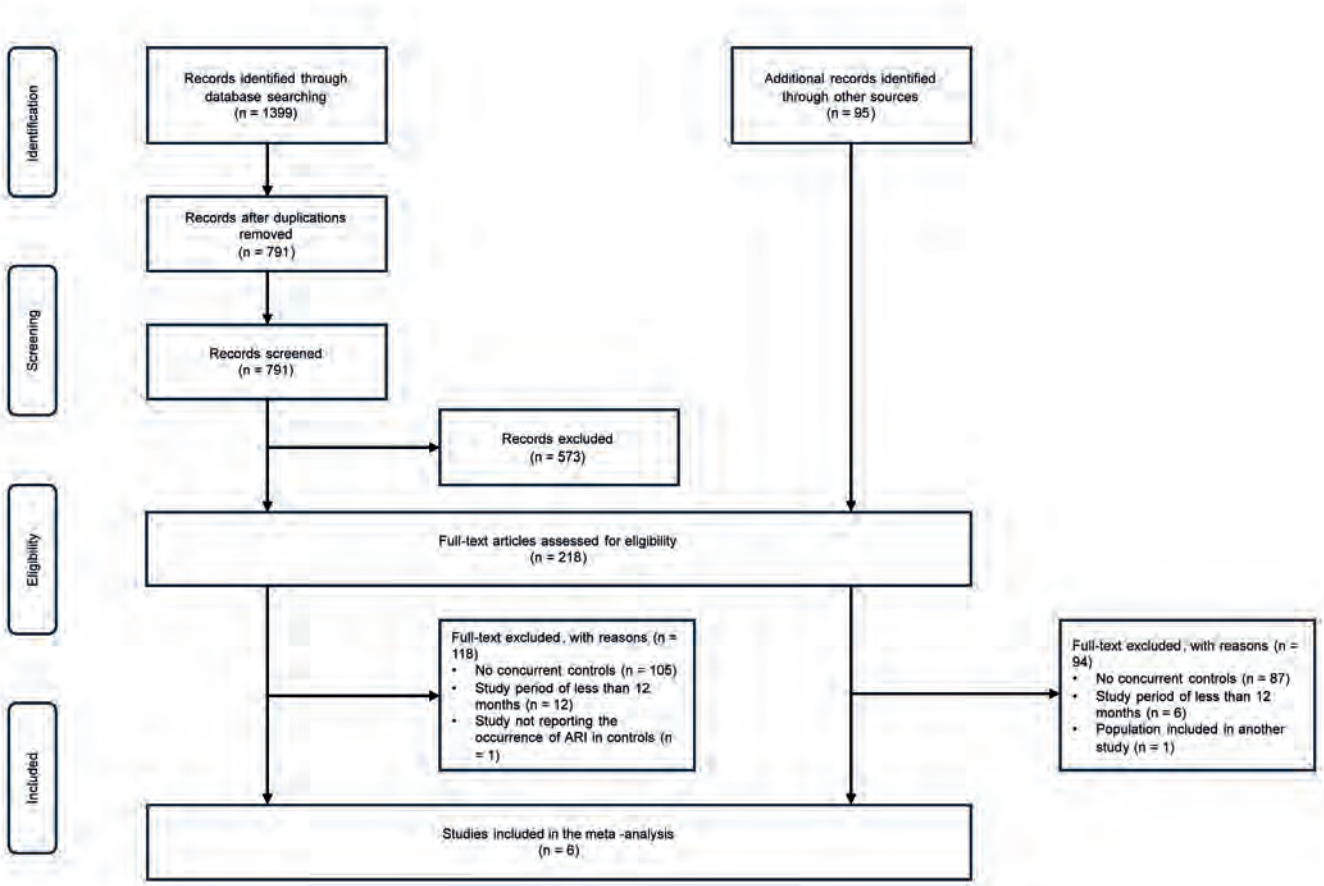


Figure 1. Flowchart of the study selection process. ARI = acute respiratory illness.

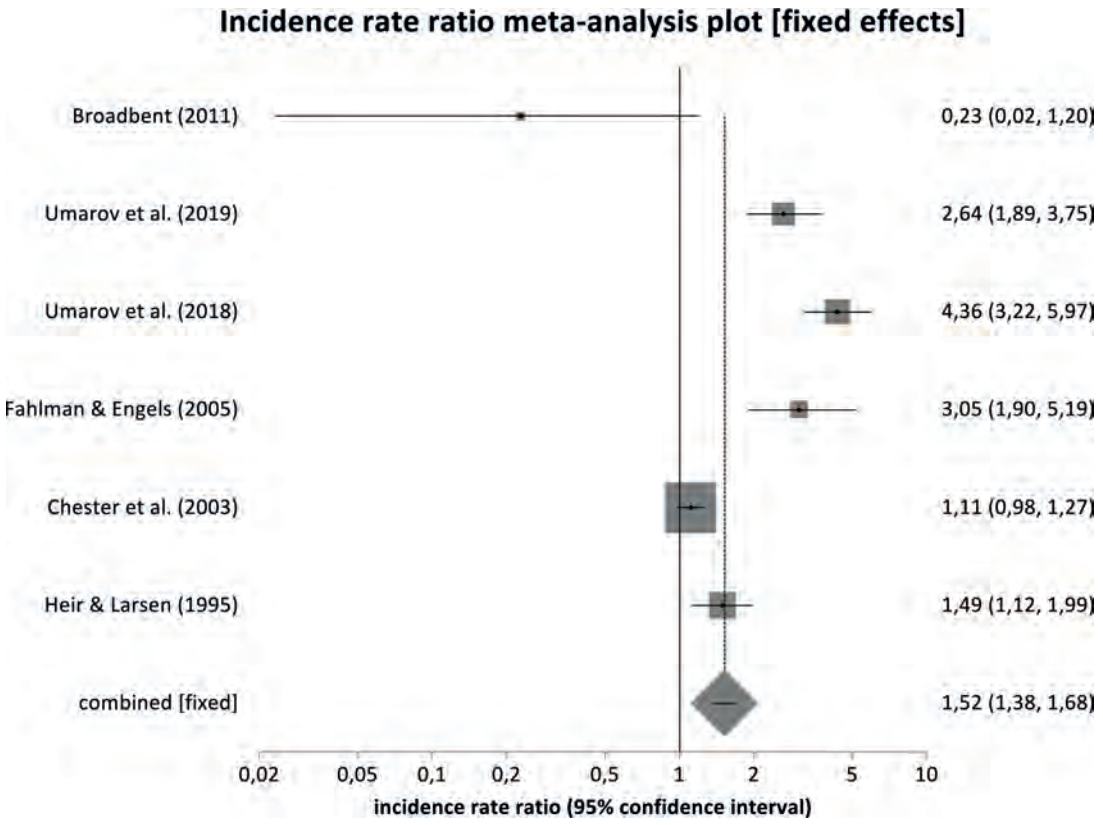


Figure 2. Pooled incidence rate ratios with 95% confidence intervals of the occurrence of ARI in athletes.

CONCLUSION

In this meta-analysis, the occurrence of ARI was significantly higher in athletes than in non-athlete controls. Most studies on the occurrence of ARIs in athletes are of low quality, and may potentially produce inaccurate assessments of the rate of ARIs. Future prospective controlled studies with a minimum duration of 12 months, with weekly monitoring and viral diagnostics, are highly needed. These kinds of studies are, however, economically and organizationally very demanding.

ACKNOWLEDGMENTS

This work was supported by the Jenny and Antti Wihuri Foundation.

AUTHORS' CONTRIBUTIONS

WG participated in the design of the study, carried out the literature search, data extraction, and analysis, and drafted the manuscript. MU confirmed the extracted data, supervised statistical analyses, and provided suggestions and revisions to the original draft. OR conceived the idea of the study, participated in the analyses, and helped to draft the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

1. Alonso J-M, Edouard P, Fischetto G, Adams B, Depiesse F, Mountjoy M. Determination of future prevention strategies in elite track and field: analysis of Daegu 2011 IAAF Championships injuries and illnesses surveillance. Br J Sports Med 46: 505–514, 2012.
2. Alonso J-M, Tscholl PM, Engebretsen L, Mountjoy M, Dvorak J, Junge A. Occurrence of injuries and illnesses during the 2009 IAAF World Athletics Championships. Br J Sports Med 44: 1100–1105, 2010.
3. Aoki, A, Ashizawa T, Ebata A, Nasu Y, Fujii T. Group A Streptococcus pharyngitis outbreak among university students in a judo club. J Infect Chemother 20: 190–193, 2014.
4. Al-Shaqsi S, Al-Kashmiri A, Al-Risi A, Al-Malawi S. Sports injuries and illnesses during the second Asian Beach Games. Br J Sports Med 46: 780–787, 2012.
5. Anglem N, Lucas SJE, Rose EA, Cotter JD. Mood, illness and injury responses and recovery with adventure racing. Wilderness Environ Med 19: 30–38, 2008.
6. Audi A, AlIbrahim M, Kaddoura M, Hijazi G, Yassine HM, Zaraket H. Seasonality of Respiratory Viral Infections: Will COVID-19 Follow Suit? Front Public Health 8: 567184, 2020.
7. Bayer C, Remschmidt C, an der Heiden M, et al. Internet-based syndromic monitoring of acute respiratory illness in the general population of Germany, weeks 35/2011 to 34/2021. Euro Surveill 19: 20684, 2014.
8. Belli A, Della Bella G, Mollo ML, Garcovich C, Foti C. Incidence of injuries and illnesses in 32(nd) America's Cup yacht racing. Medicina dello Sport 63: 239–254, 2010.
9. Bénet T, Amour S, Valette M, et al. Incidence of asymptomatic and symptomatic influenza among healthcare workers: A multicenter prospective cohort study. Clin Infect Dis 72: e311–e318, 2021.
10. Bere T, Alonso J-M, Wangensteen A, et al. Injury and illness surveillance during the 24th Men's Handball World Championship 2015 in Qatar. Br J Sports Med 49: 1151–1156, 2015.
11. Bergendiova K, Tibenska E, Majtan J. Pleuran (β-glucan from Pleurotus ostreatus) supplementation, cellular immune response and respiratory tract infections in athletes. Eur J Appl Physiol 111: 2033–2040, 2011.
12. Broadbent S. Seasonal changes in haematology, lymphocyte transferrin receptors and intracellular iron in Ironman triathletes and untrained men. Eur J Appl Physiol 111: 93–100, 2011.
13. Byington CL, Ampofo K, Stockmann C, et al. Community surveillance of respiratory viruses among families in Utah better identification of germs-longitudinal viral epidemiology (BIG-LoVE) study. Clin Infect Dis 61: 1217–1224, 2015.
14. Campbell JP and Turner JE. Debunking the myth of exercise-induced immune suppression: Redefining the impact of exercise on immunological health across the lifespan. Front Immunol 9: 648, 2018.
15. Chastin SFM, Abaraogu U, Bourgois JG, et al. Effects of regular physical activity on the immune system, vaccination and risk of community-acquired infectious disease in the general population: systematic review and meta-analysis. Sports Med 51: 1673–1686, 2021.
16. Chester N, Reilly T, Mottram DR. Over-the-counter drug use amongst athletes and non-athletes. J Sports Med Phys Fitness 43: 111–118, 2003.
17. Clancy RL, Gleeson M, Cox A, et al. Reversal in fatigued athletes of a defect in interferon gamma secretion after administration of Lactobacillus acidophilus. Br J Sports Med 40: 351–354, 2006.

18. Cohen S, Murphy MLM, Prather AA. Ten surprising facts about stressful life events and disease risk. Annu Rev Psychol 70: 577–597, 2019.
19. Cox AJ, Gleeson M, Pyne DB, Callister R, Hopkins WG, Fricker PA. Clinical and laboratory evaluation of upper respiratory symptoms in elite athletes. Clin J Sport Med 18: 438–445, 2008.
20. Cox AJ, Gleeson M, Pyne DB, Saunders PU, Callister R, Fricker PA. Respiratory symptoms and inflammatory responses to Difflam throat-spray intervention in half-marathon runners: a randomised controlled trial. Br J Sports Med 44: 127–133, 2010.
21. Crunkhorn ML, Wolff A, Drew M, et al. Establishing the incidence and prevalence of injury and illness in Australian sailing athletes over a full year of training and competition to help determine prevention priorities. J Sci Med Sport 25: 726–731, 2022.
22. Davison G, Perkins E, Jones AW, et al. Coldzyme® Mouth Spray reduces duration of upper respiratory tract infection symptoms in endurance athletes under free living conditions. Eur J Sport Sci 21: 771 - 780, 2020.
23. Derman W, Badenhorst M, Eken MM, et al. Incidence of acute respiratory illnesses in athletes: a systematic review and meta-analysis by a subgroup of the IOC consensus on 'acute respiratory illness in the athlete'. Br J Sports Med 56: 630–638 , 2022.
24. Derman W, Schwellnus M, Jordaan E. Clinical characteristics of 385 illnesses of athletes with impairment reported on the WEB-IISS system during the London 2012 Paralympic Games. PM R 6: S23–S30, 2014.
25. Derman W, Runciman P, Jordaan E, et al. Incidence rate and burden of illness at the Pyeongchang 2018 Paralympic Winter Games. Br J Sports Med 53:1099–1104, 2019.
26. Derman W, Schwellnus MP, Jordaan E, et al. Sport, sex and age increase risk of illness at the Rio 2016 Summer Paralympic Games: a prospective cohort study of 51 198 athlete days. Br J Sports Med 52: 17–23, 2018.
27. Derman W, Schwellnus MP, Jordaan E, et al. The incidence and patterns of illness at the Sochi 2014 Winter Paralympic Games: a prospective cohort study of 6564 athlete days. Br J Sports Med 50: 1064–1068, 2016.
28. Dimitriou L, Hill JA, Jehnali A, et al. Influence of a montmorency cherry juice blend on indices of exercise-induced stress and upper respiratory tract symptoms following marathon running--a pilot investigation. J Int Soc Sports Nutr 12: 22, 2015.
29. Dumontier J, Bishop NC, Vogelaers D, et al. Upper respiratory tract symptoms and salivary immunoglobulin A of elite female gymnasts: a full year longitudinal field study. Biol Sport 37: 285– 293, 2020.
30. Dvorak J, Junge A, Derman W, Schwellnus M. Injuries and illnesses of football players during the 2010 FIFA World Cup. Br J Sports Med 45: 626–630, 2011.
31. Edouard P, Depiesse F, Branco P, Alonso J-M. Analyses of Helsinki 2012 European Athletics Championships injury and illness surveillance to discuss elite athletes risk factors. Clin J Sport Med 24: 409–415, 2014.
32. Edouard P, Depiesse F, Hertert P, Branco P, Alonso J-M. Injuries and illnesses during the 2011 Paris European Athletics Indoor Championships. Scand J Med Sports 23: e213–e218, 2013.
33. Edouard P, Jacobsson J, Timpka T, et al. Extending in-competition Athletics injury and illness surveillance with pre-participation risk factor screening: A pilot study. Phys Ther Sport 16: 98–106, 2015.
34. Edouard P, Junge A, Sorg M, Timpka T, Branco P. Illnesses during 11 international athletics championships between 2009 and 2017: incidence, characteristics and sex-specific and discipline-specific differences. Br J Sports Med 53: 1174–1182, 2019.
35. Ekblom B, Ekblom O, Malm C. Infectious episodes before and after a marathon race. Scand J Med Sci Sports 16: 287–293, 2006.
36. Engebretsen L, Soligard T, Steffen K, et al. Sports injuries and illnesses during the London Summer Olympic Games 2012. Br J Sports Med 47: 407–414, 2013.
37. Engebretsen L, Steffen K, Alonso JM, et al. Sports injuries and illnesses during the Winter Olympic Games 2010. Br J Sports Med 44: 772–780, 2010.
38. Fahlman MM, Engels H-J. Mucosal IgA and URTI in American college football players: a year longitudinal study. Med Sci Sports Exerc 37: 374–380, 2005.
39. Francis JL, Gleeson M, Pyne DB, Callister R, Clancy RL. Variation of salivary immunoglobulins in exercising and sedentary populations. Med Sci Sports Exerc 37: 571–578, 2005.
40. Fricker PA, Pyne DB, Saunders PU, Cox AJ, Gleeson M, Telford RD. Influence of training loads on patterns of illness in elite distance runners. Clin J Sport Med 15: 246–252, 2005.
41. Furusawa K, Tajima F, Okawa H, Takahashi M, Ogata H. The incidence of post-race symptoms of upper respiratory tract infection in wheelchair marathon racers. Spinal Cord 45: 513–517, 2007.
42. Gallea JW, Higgins GL 3rd, Germann CA, Strout TD. Injury and illness sustained by human competitors in the 2010 iditarod sled dog race. Am J Emerg Med 32: 780–784, 2014.
43. García-Arroyo L, Prim N, Del Cuerpo M, et al. Prevalence and seasonality of viral respiratory infections in a temperate climate region: A 24-year study (1997-2020). Influenza Other Respir Viruses 16: 756–766, 2022.
44. Gawronski W, Sobiecka J, Malesza J. Fit and healthy Paralympians--medical care guidelines for disabled athletes: a study of the injuries and illnesses incurred by the Polish Paralympic team in Beijing 2008 and London 2012. Br J Sports Med 47: 844–849, 2013.
45. Geismar C, Nguyen V, Fragaszy E, et al. Symptom profiles of community cases infected by influenza, RSV, rhinovirus, seasonal coronavirus, and SARS-CoV-2 variants of concern. Sci Rep 13: 12511, 2023.
46. Gill SK, Teixeira AM, Rosado F, et al. The impact of a 24-h ultra-marathon on salivary antimicrobial protein responses. Int J Sports Med 35: 966–971, 2014.
47. Gleeson M, McDonald WA, Pyne DB, et al. Immune status and respiratory illness for elite swimmers during a 12-week training cycle. Int J Sports Med 21: 302–307, 2000.
48. Gleeson M, Pyne DB, Austin JP, et al. Epstein-Barr virus reactivation and upper-respiratory illness in elite swimmers. Med Sci Sports Exerc 34: 411–417, 2002.
49. Gleeson M, Pyne DB, Elkington LJ, et al. Developing a multi-component immune model for evaluating the risk of respiratory illness in athletes. Exerc Immunol Rev 23: 52–64, 2017.
50. Gleeson M, Williams C. Intense exercise training and immune function. Nestle Nutr Inst Workshop Ser 76: 39–50, 2013.
51. Gordon L, Schwellnus M, Swanevelder S, Jordaan E, Derman W. Recent acute prerace systemic illness in runners increases the risk of not finishing the race: SAFER study V. Br J Sports Med 51: 1295–1300, 2017.
52. Grabs V, Kersten A, Haller B, et al. Rutoside and hydrolytic enzymes do not attenuate marathon-induced inflammation. Med Sci Sports Exerc 49: 387–395, 2017.
53. Granados A, Goodall EC, Luinstra K, Smieja M, Mahony J. Comparison of asymptomatic and symptomatic rhinovirus infections in university students: incidence, species diversity, and viral load. Diagn Microbiol Infect Dis 82: 292–296, 2015.

54. Gundlapalli AV, Rubin MA, Samore MH, et al. Influenza, Winter Olympiad, 2002. Emerg Infect Dis 12: 144–146, 2006.
55. Hamid MSA, Puji A, Salleh Z, Jamalullail Z, Hussein KH. Patterns of injuries and illness among Malaysian athletes during the XVII Asian Games 2014. Sains Malaysiana 45: 1531–1536, 2016.
56. Hanstad DV, Rønsen O, Andersen SS, Steffen K, Engebretsen L. Fit for the fight? Illnesses in the Norwegian team in the Vancouver Olympic Games. Br J Sports Med 45: 571–575, 2011.
57. Hanstock HG, Govus AD, Stenqvist TB, Melin AK, Sylta Ø, Torstveit MK. Influence of immune and nutritional biomarkers on illness risk during interval training. Int J Sports Physiol Perform 15: 60–67, 2020.
58. Hausswirth C, Louis J, Aubry A, Bonnet G, Duffield R, Meur YLE. Evidence of disturbed sleep and increased illness in overreached endurance athletes. Med Sci Sports Exerc 46: 1036–1045, 2014.
59. Haywood BA, Black KE, Baker D, McGarvey J, Healey P, Brown RC. Probiotic supplementation reduces the duration and incidence of infections but not severity in elite rugby union players. J Sci Med Sport 13: 22–29, 2014.
60. Heikkinen T, Järvinen A. The common cold. Lancet 361: 51–59, 2003.
61. Heir T, Larsen S. The influence of training intensity, airway infections and environmental conditions on seasonal variations in bronchial responsiveness in cross-country skiers. Scand J Med Sci Sports 5: 152–159, 1995.
62. Hellard P, Guimaraes F, Avalos M, Houel N, Hausswirth C, Toussaint JF. Modeling the association between HR variability and illness in elite swimmers. Med Sci Sports Exerc 43: 1063–1070, 2011.
63. Hellard P, Guimaraes F, Avalos M, et al. Modeling the association between HR variability and illness in elite swimmers. Med Sci Sports Exerc 43: 1063–1070, 2011.
64. Henson D, Nieman D, Davis JM, et al. Post-160-km race illness rates and decreases in granulocyte respiratory burst and salivary IgA output are not countered by quercetin ingestion. Int J Sports Med 29: 856–863, 2008.
65. Ihalainen JK, Schumann M, Häkkinen K, Mero AA. Mucosal immunity and upper respiratory tract symptoms in recreational endurance runners. Appl Physiol Nutr Metab 41: 96–102, 2015.
66. Ip DKM, Lau LLH, Leung NHL, et al. Viral shedding and transmission potential of asymptomatic and paucisymptomatic influenza virus infections in the community. Clin Infect Dis 64: 736–742, 2017.
67. Jacobs SE, Lamson DM, St George K, Walsh TJ. Human rhinoviruses. Clin Microbiol Rev 26: 135–162, 2013.
68. Jeffries AC, Wallace L, Coutts AJ, et al. Injury, illness, and training load in a professional contemporary dance company: A prospective study. J Athl Train 55: 967–976, 2020.
69. Jin X, Ren J, Li R, et al. Global burden of upper respiratory infections in 204 countries and territories, from 1990 to 2019. EClinicalMedicine 37: 100986, 2021.
70. Juhász V, Csulak E, Szabó L, et al. Retrospective study of COVID-19 experiences in elite multinational aquatic athletes. Sci Rep 13: 13978, 2023.
71. Kim D-S, Lee Y-H, Bae KS, et al. PyeongChang 2018 Winter Olympic Games and athletes' usage of 'polyclinic' medical services. BMJ Open Sport Exerc Med 5: e000548, 2019.
72. Kon M, Iizuka T, Maegawa T, et al. Salivary secretory immunoglobulin A response of elite speed skaters during a competition period. J Strength Cond Res 24: 2249–2254, 2010.
73. Krüger K, Mooren F-C, Pilat C. The Immunomodulatory effects of physical activity. Curr Pharm Des 22: 3730–3748, 2016.
74. Lindblad N, Hänninen T, Valtonen M, Heinonen OJ, Waris M, Ruuskanen O. Influenza A outbreaks in two professional ice hockey teams during COVID-19 epidemic. Viruses 14: 2730, 2022.
75. Lipsett SC, Monuteaux MC, Fine AM. Seasonality of common pediatric infectious diseases. Pediatr Emerg Care 37: 82–85, 2021.
76. Llinás PJ, Serrano RF, Quintero Barrera L, Quiceno Noguera JC, Martinez Cano JP. Sports injuries and ill-health episodes in the Cali 2013 World Games. BMJ Open Sport Exerc Med 2: e000072, 2016.
77. Luoto R, Waris M, Valtonen M, Ruuskanen O. Respiratory viral infections - impact on sport and exercise medicine. Exerc Immunol Rev 29: 7–21, 2023.
78. Ma X, Conrad T, Alchikh M, Reiche J, Schweiger B, Rath B. Can we distinguish respiratory viral infections based on clinical features? A prospective pediatric cohort compared to systematic literature review. Rev Med Virol 28: e1997, 2018.
79. McLaughlin KA, Townes DA, Wedmore IS, Billingsley RT, Listrom CD, Iverson LD. Pattern of injury and illness during expedition-length adventure races. Wilderness Environ Med 17: 158–161, 2006.
80. Milanez VF, Ramos SP, Okuno NM, Boullosa DA, Nakamura FY. Evidence of a non-linear dose-response relationship between training load and stress markers in elite female futsal players. J Sports Sci Med 13: 22–29, 2014.
81. Monto AS, Malosh RE, Petrie JG, Thompson MG, Ohmit SE. Frequency of acute respiratory illnesses and circulation of respiratory viruses in households with children over 3 surveillance seasons. J Infect Dis 210: 1792–1799, 2014.
82. Monto AS, Sullivan KM. Acute respiratory illness in the community. Frequency of illness and the agents involved. Epidemiol Infect 110: 145–160, 1993.
83. Moreira A, Arsati F, Cury PR, et al. The impact of a 17-day training period for an international championship on mucosal immune parameters in top-level basketball players and staff members. Eur J Oral Sci 116: 431–437, 2008.
84. Moreira A, Arsati F, de Oliveira Lima-Arsati, YB, Simoes AC, de Araújo VC. Monitoring stress tolerance and occurrences of upper respiratory illness in basketball players by means of psychometric tools and salivary biomarkers. Stress and Health 27: E166–E172, 2011.
85. Moreira A, Delgado L, Moreira P, Haahtela T. Does exercise increase the risk of upper respiratory tract infections? Br Med Bull 90: 111–131, 2009.
86. Moriyama M, Hugentobler WJ, Iwasaki A. Seasonality of respiratory viral infections. Annu Rev Virol 7: 83–101, 2020.
87. Mortatti AL, Moreira A, Aoki MS, et al. Effect of competition on salivary cortisol, immunoglobulin A, and upper respiratory tract infections in elite young soccer players. J Strength Cond Res 26: 1396–401, 2012.
88. Mountjoy M, Junge A, Alonso JM, et al. Sports injuries and illnesses in the 2009 FINA World Championships (Aquatics). Br J Sports Med 44: 522–527, 2010.
89. Mäkelä MJ, Puhakka T, Ruuskanen O, et al. Viruses and bacteria in the etiology of the common cold. J Clin Microbiol 36: 539–542, 1998.
90. Nabhan D, Windt J, Taylor D, Moreau W. Close encounters of the US kind: illness and injury among US athletes at the PyeongChang 2018 Winter Olympic Games. Br J Sports Med 54: 997–1002, 2020.
91. Nakamura D, Akimoto T, Suzuki S, Kono I. Daily changes of salivary secretory immunoglobulin A and appearance of upper respiratory symptoms during physical training. J Sports Med Phys Fitness 46: 152–157, 2006.

92. Neville VJ, Molloy J, Brooks JHM, Speedy DB, Atkinson G. Epidemiology of injuries and illnesses in America's Cup yacht racing. Br J Sports Med 40: 304–312, 2006.
93. Nieman DC, Henson DA, Gross SJ, et al. Quercetin reduces illness but not immune perturbations after intensive exercise. Med Sci Sports Exerc 39: 1561–1569, 2007.
94. Nieman DC, Henson DA, McMahon M, et al. Beta-glucan, immune function, and upper respiratory tract infections in athletes. Med Sci Sports Exerc 40: 1463–1471, 2008.
95. Nieman DC, Wentz LM. The compelling link between physical activity and the body's defense system. J Sport Health Sci 8: 201–217, 2019.
96. Orysiak J, Witek K, Zembron-Lacny A, Morawin B, Malczewska-Lenczowska J, Sitkowski D. Mucosal immunity and upper respiratory tract infections during a 24-week competitive season in young ice hockey players. J Sports Sci 35: 1255–1263, 2017.
97. Pacque PFJ, Booth CK, Ball MJ, Dwyer DB. The effect of an ultra-endurance running race on mucosal and humoral immune function. J Sports Med Phys Fitness 47: 496–501, 2007.
98. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 372: n71, 2021.
99. Palmer-Green D, Elliott N. Sports injury and illness epidemiology: Great Britain Olympic Team (TeamGB) surveillance during the Sochi 2014 Winter Olympic Games. Br J Sports Med 49: 25–29, 2015.
100. Patatoukas D, Farmakides A, Aggeli V, et al. Disability-related injuries in athletes with disabilities. Folia Med (Plovdiv) 53: 40–46, 2011.
101. Pedersen A, Zachariae R, Bovbjerg DH. Influence of psychological stress on upper respiratory infection – a meta-analysis of prospective studies. Psychosom Med 72: 823–832, 2010.
102. Peters EM, Robson PJ, Kleinveldt NC, Naicker VL, Jogessar VD. Hematological recovery in male ultramarathon runners: the effect of variations in training load and running time. J Sports Med Phys Fitness 44: 315–321, 2004.
103. Peters EM, Shaik J, Kleinveldt N. Upper respiratory tract infection symptoms in ultramarathon runners not related to immunoglobulin status. Clin J Sport Med 20: 39–46, 2010.
104. Price RHM, Graham C, Ramalingam S. Association between viral seasonality and metrological factors. Sci Rep 9: 929, 2019.
105. Prien A, Mountjoy M, Miller J, et al. Injury and illness in aquatic sport: how high is the risk? A comparison of results from three FINA World Championships. Br J Sports Med 51: 277–282, 2017.
106. Pyne DB, McDonald WA, Gleeson M, Flanagan A, Clancy RL, Fricker PA. Mucosal immunity, respiratory illness, and competitive performance in elite swimmers. Med Sci Sports Exerc 33: 348–353, 2001.
107. Pyöriä L, Valtonen M, Luoto R, et al. Survey of viral reactivations in elite athletes: A case-control study. Pathogens 10: 666, 2021.
108. Renko M, Tapiainen T. Change in respiratory syncytial virus seasonality in Finland. Acta Paerdiatr 109: 202–203, 2020.
109. Robson-Ansley P, Howatson G, Tallent J, et al. Prevalence of allergy and upper respiratory tract symptoms in runners of the London Marathon. Med Sci Sports Exerc 44: 999–1004, 2012.
110. Ruedl G, Schobersberger W, Pocecco E, et al. Sport injuries and illnesses during the first Winter Youth Olympic Games 2012 in Innsbruck, Austria. Br J Sports Med 46: 1030–1037, 2012.
111. Ruohola A, Waris M, Allander T, Ziegler T, Heikkinen T, Ruuskanen O. Viral etiology of common cold in children, Finland. Emerg Infect Dis 15: 344–346, 2009.
112. Ruuskanen O, Dollner H, Luoto R, Valtonen M, Heinonen OJ, Waris M. Contract of respiratory viral infection during air travel: an underrecognized health risk for athletes. J Sport Health Sci. Accepted for publication.
113. Ruuskanen O, Luoto R, Valtonen M, Heinonen OJ, Waris M. Respiratory viral infections in athletes: many unanswered questions. Sports Med 52: 2013–2021, 2022.
114. Scherr J, Nieman DC, Schuster T, et al. Nonalcoholic beer reduces inflammation and incidence of respiratory tract illness. Med Sci Sports Exerc 44: 18–26, 2012.
115. Shaw DM, Merien F, Braakhuis A, Dulson D. T-cells and their cytokine production: The anti-inflammatory and immunosuppressive effects of strenuous exercise. Cytokine 104: 136–142, 2018.
116. Shimizu K, Suzuki N, Nakamura M, et al. Mucosal immune function comparison between amenorrheic and eumenorrheic distance runners. J Strength Cond Res 26: 1402 -1406, 2012.
117. Simpson RJ, Kunz H, Agha N, Graff R. Exercise and the regulation of immune functions. Prog Mol Biol Transl Sci 135: 355–380, 2015.
118. Soligard T, Palmer D, Steffen K, et al. Sports injury and illness incidence in the PyeongChang 2018 Olympic Winter Games: a prospective study of 2914 athletes from 92 countries. Br J Sports Med 53: 1085 - 1092, 2019.
119. Soligard T, Steffen K, Palmer-Green D, et al. Sports injuries and illnesses in the Sochi 2014 Olympic Winter Games. Br J Sports Med 49: 441–447, 2015.
120. Soligard T, Steffen K, Palmer D, et al. Sports injury and illness incidence in the Rio de Janeiro 2016 Olympic Summer Games: A prospective study of 11274 athletes from 207 countries. Br J Sports Med 51: 1265–1271, 2017.
121. Somerville V, Gill ND, Ross A, Braakhuis A. Upper respiratory illness in different tiers of rugby union. Sport Sci Health 15: 197–205, 2019.
122. Somerville V, Moore R, Braakhuis A. The effect of olive leaf extract on upper respiratory illness in high school athletes: A randomised control trial. Nutrients 11: 358, 2019.
123. Spence L, Brown W, Pyne DB, et al. Incidence, etiology, and symptomatology of upper respiratory illness in elite athletes. Med Sci Sports Exerc 39: 577–586, 2007.
124. Steffen K, Moseid CH, Engebretsen L, et al. Sports injuries and illnesses in the Lillehammer 2016 Youth Olympic Winter Games. Br J Sports Med 51: 29–35, 2017.
125. Steffen K, Soligard T, Mountjoy M, et al. How do the new Olympic sports compare with the traditional Olympic sports? Injury and illness at the 2018 Youth Olympic Summer Games in Buenos Aires, Argentina. Br J Sports Med 54: 168–175, 2020.
126. Stockwell MS, Reed C, Vargas CY, et al. Five-year community surveillance study for acute respiratory infections using text messaging: findings from the MoSAIC study. Clin Infect Dis 75: 987–995, 2022.
127. Strasser B, Geiger D, Schauer M, et al. Probiotic supplements beneficially affect tryptophan-kynurenine metabolism and reduce the incidence of upper respiratory tract infections in trained athletes: A randomized, double-blinded, placebo-controlled trial. Nutrients 8: 752, 2016.
128. Svendsen IS, Taylor IM, Tønnessen E, Bahr R, Gleeson M. Training-related and competition-related risk factors for respiratory tract and gastrointestinal infections in elite cross-country skiers. Br J Sports Med 50: 809–815, 2016.
129. Szilagyi PG, Blumkin A, Treanor JJ, et al. Incidence and viral aetiologies of acute respiratory illnesses (ARIs) in the United States: a population-based study. Epidemiol Infect 144: 2077–2086, 2016.

130. Theron N, Schwellnus M, Derman W, Dvorak J. Illness and injuries in elite football players – a prospective cohort study during the FIFA Confederations Cup 2009. Clin J Sport Med 23: 379–383, 2013.
131. Tiernan C, Lyons M, Comyns T, Nevill AM, Warrington G. Salivary IgA as a predictor of upper respiratory tract infections and relationship to training load in elite rugby union players. J Strength Cond Res 34: 782–790, 2020.
132. Tokars JI, Olsen SJ, Reed C. Seasonal incidence of symptomatic influenza in the United States. Clin Infect Dis 66: 1511–1518, 2018.
133. Tsai M-L, Chou K-M, Chang C-K, Fang S-H. Changes of mucosal immunity and antioxidation activity in elite male Taiwanese taekwondo athletes associated with intensive training and rapid weight loss. Br J Sports Med 45: 729–734, 2011.
134. Umarov J, Kerimov F, Toychiev A, et al. Association of the 25(OH) vitamin D status with upper respiratory tract infections morbidity in water sports elite athletes. J Sports Med Phys Fitness 59: 2058–2065, 2019.
135. Umarov J, Kerimov F, Toychiev A, Osipova S. Vitamin D level among elite wrestlers in Uzbekistan. Eur Sci Rev 1: 173–177, 2018.
136. Valtonen M, Grönroos W, Luoto R, et al. Increased risk of respiratory viral infections in elite athletes: A controlled study. PLoS One 16: e0250907, 2021.
137. Valtonen M, Waris M, Luoto R, et al. Non-SARS-CoV-2 Respiratory Viruses in Athletes at Major Winter Sport Events, 2021 and 2022. Emerg Infect Dis 28: 2096-2099, 2022.
138. Valtonen M, Waris M, Vuorinen T, et al. Common cold in Team Finland during 2018 Winter Olympic Games (PyeongChang): epidemiology, diagnosis including molecular point-of-care testing (POCT) and treatment. Br J Sports Med 53:1093–8, 2019.
139. van Beijsterveldt AMC, Thijs KM, Backx FJG, Steffen K, Brozicevic V, Stubbe JH. Sports injuries and illnesses during the European Youth Olympic Festival 2013. Br J Sports Med 49: 448–452, 2015.
140. Van Tonder A, Schwellnus M, Swanevelder S, Jordaan E, Derman W, Janse van Rensburg DC. A prospective cohort study of 7031 distance runners shows that 1 in 13 report systemic symptoms of an acute illness in the 8-12 day period before a race, increasing their risk of not finishing the race 1.9 times for those runners who started the race: SAFER study IV. Br J Sports Med 50: 939–945, 2016.
141. Walport MJ. Executive Summary to the Royal Society report "COVID-19: examining the effectiveness of non-pharmaceutical interventions". Philos Trans A Math Phys Eng Sci 381: 20230211, 2023.
142. Wang H, Theall BM, Early KS, et al. Seasonal changes in physiological and psychological parameters of stress in collegiate swimmers. Sci Rep 13: 10995, 2023.
143. Waris M. Pattern of respiratory syncytial virus epidemics in Finland: two-year cycles with alternating prevalence of groups A and B. J Infect Dis 163: 464–469, 1991.
144. Watanabe K, Akama T, Asakawa S, et al. Medical services at the 2017 Sapporo Asian winter games: injury and illness epidemiology at a 34-nation multisport event. Br J Sports Med 53: 32–36, 2019.
145. Wells GA, Shea B, O´Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available at: https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp
146. World Health Organization. RSV surveillance case definitions. Available at: https://www.who.int/teams/global-influenza-programme/global-respiratory-syncytial-virus-surveillance/case-definitions
147. Wright PA, Innes KE, Alton J, Bovbjerg VE, Owens JE. A pilot study of qigong practice and upper respiratory illness in elite swimmers. Am J Chin Med 39: 461–475, 2011.
148. Yamauchi R, Shimizu K, Kimura F, et al. Virus activation and immune function during intense training in rugby football players. Int J Sports Med 32: 393–398, 2011.
149. Zehsaz F, Farhangi N, Legge M. Mannose-binding lectin 2 gene polymorphism and susceptibility to upper respiratory tract infection among endurance athletes. Eur J Sport Sci 14: 586–591, 2014.

Is stretching an appropriate control for studies on exercise immunology in older adults? A systematic review.

Emelyn MATHOT[*1,2] Lene SALIMANS[*1,2], Arno GOENS[1,2], Rose Njemini[1,2], Jan Gutermuth[3,4], Inge KORTEKAAS KROHN[3,4], Ivan BAUTMANS[1,2,5, 6+]

[1] Frailty and Resilience in Ageing (FRIA) Research unit, Vitality Research group, Vrije Universiteit Brussel (VUB), Laarbeeklaan 103, 1090 Brussels, Belgium
[2] Department of Gerontology, Vrije Universiteit Brussel (VUB), Laarbeeklaan 103, 1090 Brussels, Belgium
[3] SKIN Research unit, Vitality Research group, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium
[4] Dermatology Department, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, 1090 Brussel, Brussels, Belgium
[5] Department of Geriatrics, Universitair ziekenhuis Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium
[6] SOMT University of Physiotherapy, Softwareweg 5, 3821 BN Amersfoort, The Netherlands

[*] MATHOT & SALIMANS contributed evenly and should be indexed as joint first authors

ABSTRACT

Objective: Active muscle contraction is assumed to be essential in the anti-inflammatory effect of physical exercise, also in older adults. Although stretching does not involve active muscle contractions, its (anti)inflammatory effects remain unclear. This systematic review aims to determine whether stretching affects the inflammatory profile of older adults and if it can be considered as an appropriate control intervention for exercise immunology studies.

Methods: This systematic review was registered in PROSPERO (CRD42023388920) and conducted in accordance with PRISMA guidelines. PubMed and Web of Science were systematically screened for articles describing the effect of muscle stretching on the inflammatory profile (immune cell proportions, cytokines, oxidative markers, and inflammatory gene expression in muscle/immune cells) in older adults. A methodological quality assessment was performed using the ROB2 tool and effect sizes (ES) were calculated.

Results: Nine randomized controlled trials were included, all showing sufficient methodological quality and reporting effects on basal levels of inflammation. Muscle stretching had no effect on the number of naïve, memory, and senescence-prone T-cells or circulating inflammatory markers CRP and IL-6 neither on most studied oxidative stress markers (SOD, NO, VCAM, ICAM, PTX3, OX-LDL, MDA, HNE, nitrotyrosine, ox-LDL, and protein carbonyls). However, the oxidative stress marker LPO increased (ES=0.76) while CAT, ROS, fibrinogen, and MDA-LDL decreased (ES between -0.50 and -0.63) after stretching in older persons with chronic diseases. Contradictory results were found for TNF-alpha and gene expression levels. One study observed no changes in circulating TNF-alpha after stretching in healthy women, while another study showed an increase in muscle gene expression of TNF-alpha (ES=1.60) as well as circulating TNF-alpha (ES=0.64) in men with peripheral arterial disease. Regarding gene expression changes in pro/anti-inflammatory related genes, one study analysing RNA extracted from peripheral blood mononuclear cells showed stretching-induced increases (fold change≥1.5) or decreases (fold change≤0.67), while another study using RNA from buffy coat samples demonstrated no effect on gene expression.

Conclusion: Passive or active types of muscle stretching appears to be a suitable active control for exercise immunology studies in older populations. However, in populations with peripheral artery disease stretching may affect the inflammatory profile, possibly due to a higher overall inflammatory status.

[+]*Corresponding author:*
Ivan BAUTMANS
Frailty and Resilience in Ageing Research unit,
Vitality Research group
Vrije Universiteit Brussel (VUB),
Laarbeeklaan 103, B-1090 Brussels (Belgium).
Tel.: + 32 2 477 42 07
E-mail: ivan.bautmans@vub.be (I. Bautmans),
URL: https://fria.research.vub.be/ (I. Bautmans)

INTRODUCTION

The average age and life expectancy of men and women has increased drastically, giving rise to many novel healthcare challenges. Consequently, the incidence of late-life diseases tends to increase, resulting in a higher burden on scarce healthcare resources (1, 2). Older adults possess a higher risk of infection and the occurrence of chronic diseases (3, 4). An increased level of inflammation is associated with a higher incidence of illness and death (5). The phenomenon of age-associated elevations of pro-inflammatory markers, such as interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α) and C-reactive protein (CRP), in blood and tissue is defined as 'inflammageing' (5-7). This pro-inflammatory profile that emerges during ageing can partly be explained by a shift of immune cell phenotype towards a more senescent-prone cell (SPC) composition. These senescent-prone cells contribute to an accumulation of pro-inflammatory cytokines via their senescence-associated secretory phenotype (SASP), inducing an (unresolved) state of chronic inflammation, known as chronic low grade inflammatory profile (CLIP), even without the presence of antigenic stimuli (8-10). The transition from more naïve to memory- and senescent-prone T-cells is part of a gradual remodelling of the immune system during ageing, referred to as 'immunosenescence' (8, 9, 11). Immunosenescence is among others associated with a reduced vaccination response and increased susceptibility to infections (12-14). Moreover, increases in cytokines, such as IL-6 and CRP, are associated with frailty, cardiovascular diseases, sarcopenia, and other pathophysiological conditions (6, 10, 11). Additionally, the ageing process is characterised by an increase in reactive oxygen species (ROS) and decrease in anti-oxidant systems, leading to oxidative stress that can damage various cell components, exacerbating the ageing process and contributing to ageing-related diseases (15, 16).

Recent studies showed that physical exercise can exert beneficial effects on the inflammatory status, immunosenescence and oxidative stress-related markers in older persons (17-20). It has been shown that exercise can increase the number of naïve T-cells, enhance T-cell proliferation, and decrease the number of senescent-prone CD4+ and CD8+ T-cells. These changes in the immune system could therefore (at least partially) alleviate or counteract immunosenescence (17, 21, 22). Exercise can also decrease markers of oxidative stress and improve the anti-oxidant response (19, 20). Possible mechanisms have been identified by which aerobic or resistance exercises may counteract CLIP. Firstly, physical exercise involves muscle contraction and activates energy metabolism (23), thereby stimulating skeletal muscle fibers to produce myokines which can exert anti-inflammatory effects (24). Exercise-induced production of the myokine IL-6, secreted via a TNF-independent pathway, can induce secretion of anti-inflammatory cytokines IL-1ra and IL-10 by peripheral immune cells, which inhibit the release of the pro-inflammatory cytokine IL-1β (25-27). Secondly, physical exercise induces epinephrine and norepinephrine release by the adrenal medulla and sympathetic nerves. Activation of the β adrenoceptors expressed on T- and B-cells leads to cyclic adenosine monophosphate (cAMP) production and adenyl cyclase activation. This cascade can result in IL-10 secretion by T-cells (3, 27, 28). Moreover, physical exercise can also lead to a decrease in systemic inflammation through other mechanisms, including the loss of visceral fat mass (29).

Muscle stretching exercises are defined in the Medical Subject Headings database of the National Library of Medicine as "Exercises that stretch the muscle fibers with the aim to increase muscle-tendon flexibility, improve range of motion or musculoskeletal function, and prevent injuries. There are various types of stretching techniques including active, passive (relaxed), static, dynamic (gentle), ballistic (forced), isometric, and others" (30). This form of exercise seems more suitable as an active control intervention compared to a non-exercising control in exercise intervention studies. Indeed, muscle stretching allows to control for confounding factors related to the social environment of group sessions and personalised training follow-up by instructors, but also the possible exposure to pathogens during group trainings or via public transportation to the training facility. Moreover, this type of control offers the advantage of allowing the structure, timing, and follow-up of the program, as well as the duration of individual sessions and the amount of interaction between research staff and participants, to be kept consistent across both the intervention and control arms. These elements have all been identified as potential influencers of participant motivation, study adherence and completion, and self-reported outcomes (including increased daily physical activity when being randomized to a non-exercising control group) (31, 32). Although the use of muscle stretching as an active control group for exercise interventions seems promising, the effects of muscle stretching on the inflammatory profile in older adults has not yet been documented. The suitability of muscle stretching as a control for exercise immunology studies thus remains unclear. Therefore, the aim of this systematic review was to determine whether stretching affects the inflammatory profile of older adults and if it can be considered as an appropriate control intervention for exercise immunology studies.

METHODS

Search strategy and selection criteria

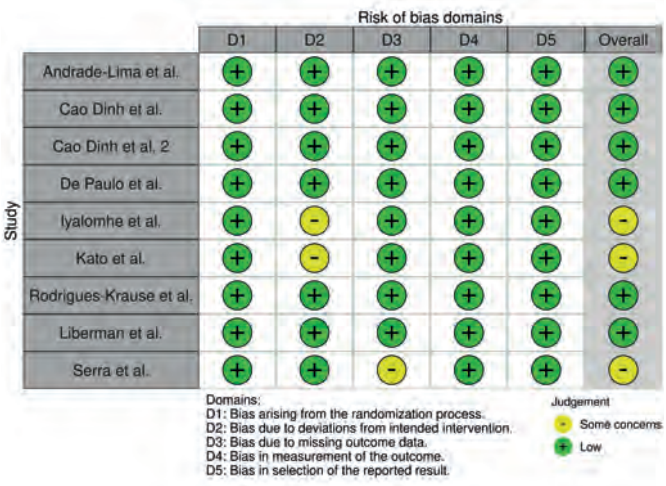
This systematic review was conducted in accordance with PRISMA guidelines, and the protocol is registered in Prospero (CRD42023388920). Two databases, PubMed and Web of Science, were systematically screened. The search key was built according to the PICO design (Population: adults aged ≥65 years old; Intervention: muscle stretching exercise; Comparison: no exercise or other forms of exercise; Outcomes: inflammatory status) including both MeSH and free terms (for the full search strings see supplement 1 & 2, for full PICO framework see Supplementary Table S1). The last search was performed on 24th of January 2024.

Study selection

Intervention studies (both randomized controlled trials (RCT),- and non-randomized controlled trials) with participants aged 65 years or older in which muscle stretching exercises were performed as experimental or control intervention were eligible for inclusion. Studies describing a combination of muscle

	P: Older adults	I: Muscle stretching	C	O: Inflammatory profile
MeSH terms	Aged	Exercise, muscle stretching Range of motion, articular		Inflammation Cytokines Immunity, cellular
Free terms	Aged Older adults Older person Older people Elderly	Muscle stretching exercise Articular range of motion "flexibility" AND "exercise" OR "flexibility" AND "training" "joint" AND "flexibility" "PNF" AND "stretching" Proprioceptive neuromuscular facilitation stretching Passive stretching Relaxed stretching Static-passive stretching Isometric stretching Active stretching Static-active stretching Ballistic stretching Dynamic stretching		Inflammation "inflammatory" AND "profile" Inflammatory Cellular immunity "immune" AND "response" "immune" AND "reaction" Cytokines Chemokine "tumor" AND "necrosis" AND "factor" "C-reactive protein"

Supplementary Table S1: Search strategy according to PICO



Supplementary Table S2: Quality assessment output – Risk of Bias

stretching with aerobic or resistance exercises, as well as yoga or Pilates, were excluded. Moreover, only studies written in English and investigating the effects of muscle stretching exercises on the inflammatory status – i.e. immune cell proportions, cytokines, oxidative stress markers, and inflammatory gene expression in muscle or immune cells - were eligible. Review articles, case reports, and animal studies were excluded. There were no restrictions on the publication date.

Firstly, two reviewers (LS and EM), who were blinded for each other's results, screened titles and abstracts for eligibility of the studies using 'Rayyan web application for systematic reviews' (33). Next, three reviewers (LS, EM and AG) performed a full-text review to further assess eligibility. A fourth reviewer (IB) was consulted to resolve disagreements.

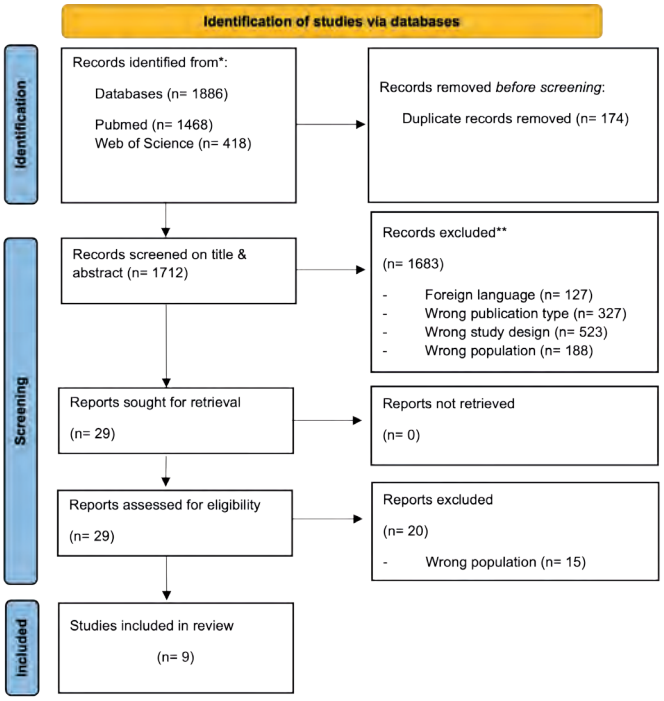


Figure 1. Flowchart Inclusion of articles

Data extraction

The main characteristics of participants (age, gender, and number of participants) and exercise interventions (training period, frequency and duration of sessions, specific type of muscle stretching exercises, number of repetitions, and duration of individual exercises) were appraised. All data regarding the inflammatory status of older adults were extracted. The outcomes were divided into three categories: 1) circulating inflammatory and oxidative stress markers (Table 1), 2) inflammation related gene expression in muscle or immune cells (Table 2), and 3) absolute counts or percentages of circulating immune cells (Table 3).This review also determined whether studies examined the effects of muscle stretching exercise on acute effects (i.e., sampling within 24h after the last exercise session) or basal levels (i.e., sampling minimum 24h after the last exercise session). The effect size (ES) of muscle stretching on outcomes of the inflammatory profile was calculated. Graph data was digitized utilizing the online tool Webplotdigitizer version 4.6 (34). When feasible, an estimate of the ES was derived following Cohen's D criteria (Small ES: d≥0.2, medium ES: d≥0.5, and large ES: d≥0.8). Only within-group comparisons were made, and the ES was determined using Hedges' average. Authors were contacted when data was unavailable. For illustrative purposes and to be able to appraise the ES of muscle stretching versus other types of (contractile) exercise, the calculated ES of muscle stretching was compared to the ES of exercise interventions extracted from recently published literature, with a preference for systematic reviews with meta-analyses or systematic reviews where ES, standardized mean difference (SMD) or weighted mean difference (WMD) were calculated. When no systematic reviews were available, recent research articles were used to compare the ES (Table 4-6).

Quality assessment of risk of bias

The quality of the included articles was assessed using the Cochrane Risk of Bias for randomized trials tool (ROB2) (35) by one reviewer (AG), which was verified by two independent reviewers (EM and LS).

RESULTS

Study selection

The medical databases PubMed and Web of Science yielded 1468 and 418 articles respectively, of which 174 articles were identified as duplicates. From these, 1683 articles were excluded after title and abstract screening due to the use of foreign languages (n=127) or wrong publication type (n=327), study design (n=523), population (n=188), intervention (n=483) or outcome (n=35) . An additional 20 articles were excluded based on full-text screening after which nine articles were included in this systematic review (Fig. 1).

Study characteristics

All included studies were randomized controlled trails (RCTs). In most studies (36-43), muscle stretching exercises were performed as an active control intervention for aerobic (36, 38, 41) or resistance (40, 42, 43) exercise interventions. De Paulo et al. (37) compared a combined aerobic with resistance program to a control muscle stretching exercise intervention. Rodrigues-Krause et al. (39) compared three interventions: a dancing intervention, an aerobic intervention, and a stretching intervention. Only one study (44) examined the effect of a stretching exercise program compared to a non-exercising control group. All studies reported 'pre-post intervention effects'. One study reported only within-group effects (44). Five studies (36, 37, 39, 42, 43) also investigated 'between group-time effects' and 'group*time effects'. One study mentioned 'within' and 'between effects' (38). Liberman et al. (40) and Iyalomhe et al. (41) reported fold changes in gene expression patterns (respectively absolute FC ≤0.67 or ≥1.5; and ≥2 considered as significant).

Participants' characteristics

In total, the studies included 163 participants. Interestingly, the effects of muscle stretching exercises on the inflammatory status of older persons were most frequently investigated in women, with 109 out of 158 participants (69%) being female. Iyalomhe et al. (41) investigated the effects in n=5 participants of both genders, however, without specifying the exact number of men and women. Most studies included healthy participants. In contrast, Andrade-Lima et al. (38) included men with peripheral artery disease (PAD) and intermittent claudication (IC) symptoms, Serra et al. (36) observed stroke survivors, Kato et al. (44) investigated chronic heart failure patients with an implantable defibrillator and De Paulo et al. (37) examined breast cancer survivors undergoing aromatase inhibitor therapy.

Intervention characteristics

The diversity in number, duration, and intensity of muscle stretching exercise sessions was high. All articles examined the effects of muscle stretching exercises on basal levels (i.e., sampling minimum 24h after the last exercise session) of inflammation. However, in one article the sampling time was not clear since the authors only mentioned that blood was taken after 12h fasting at baseline, and after 12, 24 and 36 weeks of intervention (37). Throughout these studies, the exercises consisted of similar whole body muscle stretching exercises. Four studies (38, 40, 42, 43) included passive stretching exercises for large muscle groups throughout the whole body. These muscle stretching exercises mainly induced a passive load without muscle contraction or cardiovascular challenge. Only one study described the use of active stretches (36). Four studies did not describe whether the stretching exercises was passive or active (37, 39, 41, 44). In one study (36), dynamic balance exercises were included in the stretching exercise protocol and participants received recommendations and education on cardiovascular disease risk factors and strategies to promote physical activity (36). One study included relaxation exercises in the stretching group (37), and in another study participants also performed breathing exercises (39). Heterogeneity within the frequency and duration of intervention was also observed. The shortest intervention period was 4 weeks; however, participants of this study trained every day (7x/week) (44). In another study the participants trained for 8 weeks, but only once a week (39). The longest intervention was 9 months (36 weeks), two times a week(37).

Quality assessment

All RCTs were assessed for their methodological quality. A summary of the risk of bias assessment for the nine included articles (36-44) can be retrieved in Supplementary Table 2. Six studies (37-40, 42, 43) showed a low risk of bias, while the risk of bias was of concern for three studies (36, 41, 44). Due to the nature of the interventions, participants and supervisors were aware of their assigned intervention group in all studies. Six studies (37-40, 42, 43) extensively described participant follow-up and withdrawal, unlike the other studies (41, 44), which did not address deviations or intervention compliance, resulting in some concerns regarding risk of bias. In one article, information on missing values or drop-outs was not mentioned (36).

Muscle stretching exercise effects on the inflammatory profile in older adults

Effect sizes of the muscle stretching effects were calculated and are shown in Table 4, 5 and 6. In general, most of the effect sizes for muscle stretching were small to medium.

Muscle stretching exercise effects on circulating cytokines, cell adhesion and oxidative stress markers in older adults (Table 1 and 4)

Muscle stretching showed no significant effects on circulating levels of CRP (36-39, 44) or IL-6 (36, 38). after muscle stretching. In contrast, muscle stretching increased circulatory TNF-α concentrations ($P<0.05$) in men after a 12-week training program (38), but not in women after an 8-week program (39).

Three studies (36, 38, 44) investigated the effect of muscle stretching on oxidative stress markers in older adults, two of which also examined its impact on cell adhesion markers (38, 44). Andrade-Lima et al. (38) observed no differences for plasma vascular cell adhesion molecule 1 (VCAM 1), intercellular adhesion molecule (ICAM), nitric oxide (NO) and superoxide dismutase (SOD) between baseline and post-intervention values. However, a decrease in catalase (CAT) and an increase in lipid peroxidation (LPO) was observed after stretching ($P<0.05$). Moreover, the effect on muscle related markers SOD, LPO and CAT was investigated in homogenized muscle tissue, revealing an increase in LPO ($P<0.05$), no effects on in CAT ($P > 0.05$), and no effect on SOD ($P>0.05$). In the study of Kato et al. (44) no difference in circulating pentraxin 3 (PTX3) was found after four weeks of daily stretching interventions, while a decrease of fibrinogen, malondialdehyde-modified low-density lipoprotein cholesterol (MDA-LDL), and ROS was observed ($P<0.05$). Fibrinogen and PTX3 were measured as vascular inflammation

Table 1. Effects of muscle stretching exercise on circulatory interleukins, cytokines, and oxidative stress markers

Article ID	Study design	Participants	Intervention	Group type	Acute/basal effects	Sample type	Assessment method	Outcome
Andrade-Lima et al. (2021)	RCT	Men with PAD and IC symptoms (n = 16, 69 ± 3 years[a])	2 to 3 sets of 20 stretching exercises for all body segments. These exercises were executed in 30 minutes with a passive technique and maximal stretch was maintained for 20s (2x/week for 12 weeks)	Intervention control group (for aerobic exercise intervention)	Basal effects pre-post training period	Blood plasma	ELISA (CRP, IL-6, TNF-α, VCAM, ICAM SOD, CAT, LPO) and chemiluminescence (NO)	CRP ↔ ES=0.40, IL-6 ↔ ES=0.01, TNF-α ↑ ES=0.64, VCAM ↔ ES=0.68, ICAM ↔ ES=0.34, NO ↔ ES=-1.29, SOD ↔ ES=-0.67, CAT ↓ ES=-0.63, LPO ↑ ES=0.76
						Muscle biopt	Inhibition of xanthine/xanthine oxidase driven cytochrome c (SOD), rate of H2O2 decomposition (CAT), index of oxidative injury following FOX2 (LPO)	SOD ↔ ES=-0.24, CAT ↔ ES=-0.23, LPO ↑ ES=1.11.
De Paulo et al. (2018)	RCT	Postmenopausal breast cancer survivors undergoing aromatase inhibitor therapy (n = 18, 66.6 ± 9.6 years[b])	45-minute sessions of stretching and relaxation exercises in seated or lying position. Each exercise lasted 10-15s and were selected so little stimulus was applied to the musculoskeletal system (2x/week for 36 weeks)	Intervention control group (for resistance plus aerobic exercise intervention)	No specific information - Effects pre-, 12 weeks, 24 weeks, and post training period	Blood serum	ELISA (CRP)	CRP ↔ pre-, 12 weeks ES=0.21, 24 weeks ES=-0.15, and 36 weeks ES=-0.15 post int.
Kato et al. (2017)	RCT	Patients (80% male) with chronic heart failure (n = 25, 70 ± 11 years[b])	2 sets of 7 stretches, without pain, were performed in 20 minutes: wrist dorsiflexion, wrist palmar flexion, trunk rotation, close-legged trunk flexion, open-legged trunk flexion, hip extension, and ankle dorsiflexion. Each stretch was followed by 20s of relaxation (7x/week for 4 weeks)	Intervention group (control sedentary group)	Basal effects pre-post training period	Blood serum	ELISA (hs-CRP, PTX3, fibrinogen, ROS, MDA-LDL)	hs-CRP ↔ ES=0.21, PTX3 ↔ ES=0.08, fibrinogen ↓ ($p < 0.01$) ES=-0.60, ROS ↓ ($p < 0.01$) ES=-0.59, MDA-LDL ↓ ES=-0.50
Rodrigues-Krause et al. (2018)	RCT	Women who were not engaged in any type of regular exercise training during the last 6 months (n = 10, 61-70 years[c])	2 sets of stretching exercises were executed standing or on the floor: lateral flexion of cervical spine, horizontal adduction of shoulder, knee flexion, calf stretch, and breathing exercises. Each position was maintained for 10s and sessions lasted for 60 minutes (1x/week for 8 weeks)	Intervention control group (for dancing intervention and aerobic exercise intervention)	Basal effects pre-post training period	Blood plasma	ELISA (CRP, TNF-α)	hs-CRP ↔, TNF-α ↔.
Serra et al. (2022)	RCT	Chronic stroke survivors > 6 months with self-reported mild to moderate hemiparetic gait (n = 19: 13 men and 6 women), 68 ± 2 years[d])	3 sets of 30-60 seconds of static, active stretches for neck, triceps, posterior deltoids/rhomboids, hip flexors/extensors/adductors, and gastrocnemius/soleus performed supine or seated. 3 sets of balance exercises were also executed: seated shoulder shrugs and circles, standing weight shifts, tandem/semi-tandem stance progressing to single-leg stance, heel/toe walking, and standing calf raises. Each session lasted 50 minutes (2x/week for 6 months). General recommendations and education aimed at promoting physical activity were also provided.	Intervention control group (for aerobic exercise intervention)	Basal effects - Effects pre-post training period	Blood plasma	Beckman AU480 chemistry analyzer (hs-CRP, IL-6) and ELISA (nitrotyrosine, ox-LDL, protein carbonyls, HNA and MDA adducts)	hs-CRP ↔ ES=-0,14, IL-6 ↔ ES=0,35, nitrotyrosine ↔ ES=0,02, ox-LDL ↔ ES=-0,08, protein carbonyls ↔ ES=-0,06, HNE adducts ↔ ES=0,04, MDA adducts ↔ES= 0,16.

↑: significant increase ($P < 0.05$), ↓: significant decrease ($P < 0.05$), ↔: no significant differences observed, CAT: catalase, CRP: c-reactive protein, ELISA: enzyme-linked immunosorbent assay, FOX2: ferrous oxidation xylenol orange technique, HNE: hydroxynonenal, hs-CRP: high sensitive c-reactive protein, IC: intermittent claudication, ICAM: intercellular adhesion molecule, int: intervention, IL-6: interleukin-6, ox-LDL: oxidized low-density lipoprotein, LPO: lipid peroxidation, MDA: malondialdehyde, MDA-LDL: serum malondialdehyde-modified low-density lipoprotein cholesterol, NO: nitric oxide, PAD: peripheral artery disease, PTX3: plasma pentraxin 3, RCT: randomized controlled trial, ROS: Reactive oxygen species, SOD: superoxide dismutase, TNF-α: tumor necrosis factor α, VCAM: vascular cell adhesion molecule 1.

[a] Age in mean ± SE. [b] Age in mean ± SD. [c] Age in range. [d] Age in mean ± SEM. Acute effects: sampling within 24h after the last exercise session, basal levels: sampling minimum 24h after the last exercise session

Table 2. Effects of muscle stretching exercise on inflammatory related gene expression

Article ID	Study design	Participants	Intervention	Group type	Acute/basal effects	Sample type	Assessment method	Outcome
Andrade-Lima et al. (2021)	RCT	Men with PAD and IC symptoms (n = 16, 69 ± 3 years[a])	2 to 3 sets of 20 stretching exercises for all body segments. These exercises were executed in 30 minutes with a passive technique and maximal stretch was maintained for 20s (2x/week for 12 weeks)	Intervention control group (for aerobic exercise intervention)	Basal effects pre-post training period	Muscle biopsy: mRNA samples of tissue samples from gastrocnemius muscle of the leg	Real time PCR (CRP, IL-6, TNF-α, VCAM, ICAM)	CRP ↔ ES=-0.25, IL-6 ↔ ES=0.00, TNF-α ↑ ES=1.60, VCAM ↑ ES=3.77, ICAM ↑ ES=2.67
Iyalomhe et al. (2015)	RCT	Older adults with mild cognitive impairment (n = 5, 70 ± 9 years[a])	Training consisted of static maintaining exercise positions (15-30s) to produce a slight pull on muscles, but not induce pain. Stretches were directed at tight muscles (e.g., hamstrings, hip flexors, calves, and chest) and were repeated 3-5 times for a total of about 40 minutes (3x/week for 6 months)	Intervention control group (for aerobic exercise intervention)	Basal effects pre-post training period	Heparinized Blood: RNA-samples from Buffy coat samples	Micro-array analysis	Training-induced gene expression profile ↔ (fold change ≥ 2 at p < 0.01)
Liberman et al. (2022)	RCT	Women who did not regularly perform physical exercises at higher intensities than habitual daily activity within the past 6 months (n = 5, 69,45 +/-2,62 years[a])	3 sets of 30s passive, static stretching exercises of large muscle groups were performed, mainly inducing a passive load on muscles without causing muscle contractions or cardiovascular challenge (3x/week for 3 months)	Intervention control group (for intensive strength training group IST and strength endurance training group SET)	Basal effects pre-post training period	Blood: RNA-samples of peripheral mononuclear blood cells (PBMC)	RNA-sequencing (Illumina NovaSeq 6000 system)	23 pro-inflammatory genes ↑, 9 pro-inflammatory genes ↓, 6 anti-inflammatory genes ↑, 2 anti-inflammatory genes ↓. 1 gene with unknown inflammatory profile ↑, 3 genes with unknown inflammatory profile ↓

↑: significantly upregulated (P < 0.05), ↓: significantly downregulated (P < 0.05), ↔: no significant differences observed, CAT: catalase, CRP: c-reactive protein, ICAM: intercellular adhesion molecule, eNOS: endothelial nitric oxide synthase, IL-6: interleukin-6, int.: intervention, LPO: lipid peroxidation, NO: nitric oxide, RCT: randomized controlled trial, SOD: superoxide dismutase, TNF-α: tumor necrosis factor α, VCAM: vascular cell adhesion molecule 1

[a] Mean ± standard deviation.

Acute effects: sampling within 24h after the last exercise session, basal levels: sampling minimum 24h after the last exercise session

Table 3. Effects of muscle stretching exercise on absolute cell count or percentage of T-cell subsets

Article ID	Study design	Participants	Intervention	Group type	Acute/basal effects	Sample type	Assessment method	Outcome
Cao Dinh et al. (2019)	RCT	CMV+ and CMV- women who did not regularly perform physical exercises at higher intensities than habitual daily activity within the past 6 months (n = 32, 65 years or more)	3 sets of 30s passive, static stretching exercises of large muscle groups were performed, mainly inducing a passive load on muscles without causing muscle contractions or cardiovascular challenge (2-3/week for 6 weeks)	Intervention control group (for intensive strength training group and strength endurance training group)	Basal effects pre-post training period	EDTA anticoagulated blood; Blood serum	Flow cytometry analysis (percentage of T-cell subtypes) and Cell-Dyn Sapphire haematology analyser (absolute counts)	#/% CD8+ naive T-cell subset (CD28+CD57-) ↔ ES=0.18/ES=-0.06, #/%memory CD8+ T-cell subset (CD28-CD57-) ↔ ES=0.28/ES=0.11, #/%SPC CD8+ T-cell subset (CD57+) ↔ ES=0.06/ES=-0.04), #/%SPC CD8+ T-cell subset (CD28-CD57+) ↔ ES=0.07/ES=-0.04), #/%SPC CD8+ T-cell subset (CD28+CD57+) ↔ ES=-0.04/ES=-0.03), #/% naive CD8- subset (CD28+CD57-) ↔ ES=0.45/ES=-0.01, #/% memory CD8- T-cell subset (CD28-CD57-) ↔ ES=0.06/ES=0.15, #/% SPC CD8- T-cell subset (CD57+) ↔ ES=-0.17/ES=-0.15, #/%SPC CD8+ T-cell subset (CD28-CD57+) ↔ ES=-0.17/ES=-0.18), #/%SPC CD8+ T-cell subset (CD28+CD57+) ↔ ES=-0.09/ES=-0.02) (for CMV+)
Cao Dinh et al. (2018)	RCT	CMV+ and CMV- women who did not regularly perform physical exercises at higher intensities than habitual daily activity within the past 6 months (n = 33 70.31 ± 5.15 years)	3 sets of 10-12 (30s) passive, static stretching exercises of large muscle groups were performed, mainly inducing passive load on muscles without causing muscle contractions or cardiovascular challenge (2-3/week for 6 weeks)	Intervention control group (for intensive strength training group and strength endurance training group)	Basal effects pre-post training period	EDTA anticoagulated blood; Blood serum	Flow cytometry analysis (percentage of T-cell subtypes) and Cell-Dyn Sapphire haematology analyser (absolute counts)	#/% naïve CD8+ T-cell subset (CD28+CD57-) ↔ ES=0.23/ES=-0.02, #/% memory CD8+ T-cell subset (CD28-CD57-) ↔ ES=0.19/ES=0.03, #/%SPC CD8+ T-cell subset (CD57+) ↔ ES=0.09/ES=-0.07, #/%SPC CD8+ T-cell subset (CD28-CD57+) ↔ ES=0.09/ES=-0.07, #/%SPC CD8+ T-cell subset (CD28+CD57+) ↔ ES=0.03/ES=-0.03, #/% naïve CD8- T-cell subset (CD28+CD57-) ↔ ES=0.16/ES=-0.02, #/%memory CD8- T-cell subset (CD28-CD57-) ↔ES=0.09/ES=0.10, #/% SPC CD8- T-cell subset (CD57+) ↔ ES=-0.05/ES=-0.08, #/%SPC CD8- T-cell subset (CD28-CD57+) ↔ ES=-0.06/ES=-0.10, #/%SPC CD8- T-cell subset (CD28+CD57+) ↔ ES=0.03/ES=0.07, (for both CMV+ and CMV- population)

↑: significant increase ($P < 0.05$), ↓: significant decrease ($P < 0.05$), ↔: no significant difference observed., #: absolute counts; %: percentages, CMV: cytomegalovirus serostatus (+ = positive; - = negative), EDTA: Ethylenediamine tetra acetic acid, int: intervention, RCT: randomized controlled trial, SPC: senescence-prone T-cells (CD8+/CD8-CD57+, CD8+/CD8-CD28-CD57+, CD8+/CD8-CD28+CD57+).
Acute effects: sampling within 24h after the last exercise session, basal levels: sampling minimum 24h after the last exercise session

Table 4. Overview of the effect sizes related to the exercise effects on circulatory interleukins, cytokines, and oxidative stress markers after a flexibility intervention calculated where available

↑: significantly upregulated ($P < 0.05$), ↓: significantly downregulated ($P < 0.05$), ↔: no significant differences, *Flexibility training*, *Resistance exercise*, *Aerobic exercise*, *Combined training*, (m) muscle biopsy, * SMD, ° WMD, a. Bautmans et al., 2021, Exp. Gerontology; 146: 111236. b. Salimans et al., 2022, Exp Gerontology; 164, 111822. c. Lin et al., 2015, J. Am. Heart Assoc.; 4. d. Thompson et al., 2020, J Sports Sci; 38: 814-826 e. Koh et al., 2018, J Inflamm Res; 11:296-306. f. Powers et al, 2023, Antioxidants (Basel); 12: 39., g. Ye et al., 2021, Front Physiol; 12: 701151. h. Wang et al., 2023, Open Life Sci.; 18: 668. ., j. Radak et al., 2003, **Eur J Clin Invest; 33: 726-730.** k. Zempo-Miyaki et al., 2016, J Hum Hypertens; 30: 521-526 l. **Takashima et al. 2014, Circ J.; 78: 2682-2687** , m: Kawamoto et al. 2015, Endocrine; 48: 871-877 **Bold arrows** represent populations with chronic diseases, negative effect sizes indicate smaller expression or levels after the intervention compared pre-intervention. CRP: C-reactive protein, ICAM: intercellular adhesion molecule, IL-6: interleukin-6, LPO: lipid peroxidation, MDA: malondialdehyde, MDA-LDL: serum malondialdehyde-modified low-density lipoprotein cholesterol, NO: nitric oxide, Ox-LDL: oxidized low-density lipoprotein, PTX3: plasma pentraxin 3, ROS: reactive oxygen species, SOD: superoxide dismutase, TNF-α: tumor necrosis factor α, VCAM: vascular cell adhesion molecule 1

Biomarker		Large	Medium	Small	Negligible	Small	Medium	Large
	NO ES	**ES ≤ -0,8**	**-0.8 < ES ≤ -0.5**	**-0.5 < ES ≤ -0.2**	**-0.2 < ES < 0.2**	**0.2 ≤ ES < 0.5**	**0.5 ≤ ES < 0.8**	**ES ≥ 0.8**
CRP	↓a, ↓a, ↓a, ↓a, ↔, ↔a			↓a,↓a, ↔,↓a	↔	↔,↔,↔,↔, ↔a		
IL6	↑a, ↓a, ↓a			↓a	↔	↔a, ↔	↔a, ↔a	
TNF alfa	↔, ↓a, ↓a, ↓a	↓a	↓a		↔b	↔a	↔	
Catalase	↑a		→	↔ (m)				
Fibrinogen			↓, ↓*d,↓*d	↓ °c↓°c				
ROS	↔b		→					
MDA-LDL	↓l, ↓m		→					
VCAM	↓e, ↔e, ↔e						↔	
ICAM	↓e, ↔e, ↔e					↔		
SOD	↓(m) f		↔	↓a		↔ (m)	↑*g	
LPO		↓*g						↑, ↑(m)
MDA		↓*g,↓*h, ↓*h	↓*h			↔		
NO	↔							↑*g
Nitrotyrosine	↑j				↔			
Protein carbonyls	↑j				↔			
PTX3	↑k				↔			
ox-LDL		↔*g			↔			
HNE					↔			

Table 5. Effects of muscle stretching exercise on inflammatory related gene expression

Biomarker	NO ES	<-1	-1	-0.9	-0.8	-0.7	-0.6	-0.5	-0.4	-0.3	-0.2	-0.1	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	>1.0
CRP*										↔														
IL6*													↔											
TNF alfa*																								↑
VCAM*																								↑
ICAM*																								↑
eNOS*																↔								
TIMM23	↔																							
APOBEC3C	↔																							
S100A12	↔																							
C1D	↔																							
CHMP5	↔																							
PCMTD2	↔																							
LYZ	↔																							
SNORD115_25	↔																							
IL1A	↑																							
CX3CL1	↑																							
IL1B	↑																							
CXCL2	↑																							
CCL3	↑																							
EREG	↑																							
BDKRB1	↑																							
THY1	↑																							
LTB4R2_A	↓																							
KLK15	↓																							
CD68	↑																							
RGS1	↑																							
CPA2	↓																							
REN	↔																							
CCL19	↔																							
PTGS2	↔																							
PGK1	↔																							
NCAM1	↔																							
COL3A1	↔																							
IL1RL1	↔																							
CCL2	↑																							
PLA2G5	↑																							
CXCL10	↑																							
CCL4	↑																							
DNM1P46	↓																							
SLC2A4	↓																							
HIF3A	↓																							
LRP2	↓																							
BDKRB2	↓																							
IL17A	↓																							
TERC	↑																							
CXCL11	↑																							
CD160	↔																							
KLK3	↔																							
LTC4S	↔																							
PTGER3	↔																							
CRISP3	↔																							
CCR3	↔																							
COL1A1	↔																							
LTB4R2	↔																							
NR4A2	↔																							
EPHB2	↔																							
IL5	↔																							
PDE4C	↔																							
TREM1	↔																							
IL1R2	↔																							
DUSP1	↔																							
COL4A1	↔																							
CSF3R	↔																							
CXCR1	↔																							
BMX	↔																							
MMP9	↔																							
PRKAA2	↔																							
IL3	↔																							
KLK2	↑																							
IL6	↑																							
HTR3B	↑																							
PLA2G2D	↑																							
CD69	↑																							
PLA2G2A	↑																							
TNFSF10	↑																							
IL1RAPL2	↑																							
IL9	↑																							
NFKBIA	↑																							
MXRA5	↓																							
FRZB	↓																							
HSPA1A	↔																							
FOXP3	↔																							
PTGIS	↔																							
FCGR3B	↑																							
IL13	↑																							
HRH1	↔																							
FGF21	↔																							
IL4	↔																							
ARNT2	↔																							
ASPN	↔																							
CSF3	↔																							
THBD	↔																							
HSPA1B	↔																							
SOD2	↔																							
HSPA1L	↔																							
CA4	↔																							
HSPA6	↔																							
AGTR1	↔																							
CSF2	↔																							
IGF2	↔																							
IL2	↔																							
FGF6	↔																							
SOCS3	↑																							
CACNA2D1	↑																							
CYP1A2	↓																							
MYH6	↓																							
PRM1	↔																							
RPL3L	↔																							
PTGFR	↔																							
DAAM2	↔																							
CYP7A1	↔																							
OLIG2	↓																							

↑: significantly upregulated ($P < 0.05$), ↓: significantly downregulated ($P < 0.05$), ↔: no significant differences, *Flexibility training* **Bold arrows** represent populations with chronic diseases, negative effect sizes indicate smaller expression or levels after the intervention compared pre-intervention. CRP: C-reactive protein, ICAM: intercellular adhesion molecule, IL-6: interleukin-6, TNF-α: tumor necrosis factor α, VCAM: vascular cell adhesion molecule 1, No ES were calculated because of fold change values, the fold changes ≤0.67 or ≥1.5 were clinically relevant, with a downregulation at ≤0.67 and an upregulation at ≥1.5, values between 0.67 and 1.5 were indicated as no effect. *in muscle.

Table 6. Overview of the effect sizes related to the exercise effects on absolute cell count or percentage of T-cell subsets

↑: significantly upregulated ($P < 0.05$), ↓: significantly downregulated ($P < 0.05$), ↔: no significant differences, Flexibility training, Resistance exercise, Aerobic exercise, Combined training, (m) muscle biopsy, ***Bold arrows*** *represent populations with chronic diseases, negative effect sizes indicate smaller expression or levels after the intervention compared pre-intervention., SPC: senescent-prone cell*

Cell subsets																							
	NO ES	<-1	-1	-0.9	-0.8	-0.7	-0.6	-0.5	-0.4	-0.3	-0.2	-0.1	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0	>1.0
# Naive CD8+ T-cell (CD28+CD57-) subset	↔, ↔, ↔, ↔														↔ ↔									
% Naive CD8+ T-cell (CD28+CD57-) subset	↔, ↔, ↔, ↔											↔	↔											
# memory CD8+ T-cell (CD28-CD57-) subset	↔, ↔, ↔, ↔														↔	↔								
% memory CD8+ T-cell (CD28-CD57-) subset	↔, ↔, ↔, ↔												↔	↔										
# SPC CD8+ T-cell (CD8+CD57+) subset	↓,↓, ↔, ↔													↔ ↔										
% SPC CD8+ T-cell (CD8+CD57+) subset	↓,↓, ↔, ↔											↔	↔											
# SPC CD8+ T-cell (CD8+CD28-CD57+) subset	↓, ↓, ↔, ↔													↔ ↔										
% SPC CD8+ T-cell (CD8+CD28-CD57+) subset	↓, ↓, ↔, ↔											↔	↔											
# SPC CD8+ T-cell (CD8+CD28+CD57+) subset	↔, ↓, ↔, ↔												↔ ↔											
% SPC CD8+ T-cell (CD8+CD28+CD57+) subset	↔, ↔, ↔, ↔												↔ ↔											
# naïve CD8- T-cell (CD28+CD57-) subset	↔, ↔, ↔, ↔														↔			↔						
% naïve CD8- T-cell (CD28+CD57-) subset	↔, ↔, ↔, ↔												↔ ↔											
# memory CD8- T-cell (CD28-CD57-) subset	↔, ↔, ↔, ↔													↔ ↔										
% memory CD8- T-cell (CD28-CD57-) subset	↔,↔, ↔, ↔													↔	↔									
# SPC CD8- T-cell (CD8-CD57+) subset	↔,↓, ↔, ↔										↔	↔												
% SPC CD8- T-cell (CD8-CD57+) subset	↓,↓, ↔, ↔										↔	↔												
# SPC CD8- T-cell (CD8-CD28-CD57+) subset	↔, ↔, ↔, ↔										↔	↔												
% SPC CD8- T-cell (CD8-CD28-CD57+) subset	↓, ↓, ↔, ↔										↔	↔												
# SPC CD8- T-cell (CD8-CD28+CD57+) subset	↓, ↔, ↔, ↔											↔	↔											
% SPC CD8- T-cell (CD8-CD28+CD57+) subset	↓, ↔, ↔, ↔												↔	↔										

parameters (44). Serra et al. (36) assessed blood plasma levels of nitrotyrosine, oxidized low-density lipoprotein (ox-LDL), protein carbonyls, hydroxynonenal (HNE) and malondialdehyde (MDA) adducts, but no significant effects were identified.

Muscle stretching exercise effects on inflammation related gene expression (Table 2 and 5)

Andrade-Lima et al. (38) investigated gene expression changes in muscle tissue after 12 weeks stretching intervention. No change could be observed for CRP, IL-6, and eNOS, while an increase in TNF-α, VCAM and ICAM was observed post-intervention. Iyalomhe et al. (41) analyzed changes in training-induced gene expression profile after six months of stretching intervention in RNA purified from blood samples. This study found six unique (TIMM23, APOBEC3C, S100A12, C1D, CHMP5, PCMTD2) and two non-unique (LYZ, SNORD115_25) non-overlapping genes differentially expressed after stretching intervention (fold change ≥1.5), however these changes were not statistically significant (P>0.01). The study conducted by Liberman et al. (40) investigated the effects of a 3-month stretching intervention on 407 inflammation related genes in peripheral blood mononuclear cells. In total, 23 pro-inflammatory genes were upregulated (fold change ≥1.5) and nine were downregulated (fold change ≤0.67). Similarly, six anti-inflammatory genes were upregulated (fold change ≥1.5) and two were downregulated (fold change ≤0.67) after the intervention compared to baseline expression. Additionally, this study found expression changes for four genes of which the inflammatory roles have not yet been fully determined (40).

Muscle stretching exercise effects have no effect on absolute T-cell count and percentage of T-cell subsets (Table 3 and 6)

Two studies investigated the effects of a six-week stretching intervention on absolute T-cell counts and percentage of T-cell subsets (42, 43). A distinction was made between CD8+ and CD8- T-cell subsets, which were further subdivided into three separate groups: a naïve T-cell subpopulation (CD28+CD57-), a memory T-cell subpopulation (CD28-CD57-), and a senescent-prone T-cell subpopulation (CD28-CD57+ or CD28+CD57+). No significant changes in absolute cell count or percentage of different T-cell subsets were observed after the stretching intervention, regardless of the participants' cytomegalovirus (CMV) serostatus (42).

GENERAL DISCUSSION

In this systematic literature review, we verified the suitability of muscle stretching as an appropriate control group for exercise immunology studies by appraising the impact of muscle stretching on inflammation in an older population. Traditionally, non-active or usual care groups have often been used as controls in exercise immunology studies. However, whether these non-active groups adequately represent and account for the social and physical environment intrinsically linked to exercise interventions can be questioned. These include possible confounding effects of the social environment, such as training in group settings or receiving attention from instructors, as well as effects of the physical environment, such as pathogen exposure that may arise from training in a group setting or taking (public) transport to the training facilities. Muscle stretching is considered as a suitable control intervention because it includes all the conditions of an exercise intervention group, but without (intensive) active muscle contractions. However, using muscle stretching as a control group for exercise immunology studies implies that stretching does not affect the inflammatory profile.

All articles described the long-term effects (≥24 hours after the last training session) of muscle stretching exercise programs on basal levels of inflammation. Those studies investigating the concentration of CRP (36-39, 44) and IL-6 (36, 38) in serum or plasma, observed no changes after a muscle stretching training period. In contrast, resistance and aerobic training decreases CRP-levels after long-term training. In general, the ES for resistance and for aerobic exercise are greater than the ES for muscle stretching, suggesting the appropriateness of stretching as a control intervention for those exercise programs (Table 4-6). However, Andrade-Lima et al. (38) reported a significant increase of circulatory TNF-α with an ES of 0.64 after 12 weeks (2x/week) stretching in older men with PAD or IC. This was not found by Rodrigues-Krause et al. (39) in healthy older women after muscle stretching (combined with breathing exercises) once a week for 8 weeks. These differences might be explained by an increased baseline inflammatory status of patients with PAD or IC, as inflammation contributes to disease initiation and progression(45). Indeed, Signorelli et al. previously showed that patients suffering from PAD exhibit higher resting levels of TNF-α compared to healthy controls(46). In PAD patients, higher TNF-α levels were associated with lower ankle-brachial index levels, reflecting a higher disease severity(47). Therefore, the increase in TNF-α concentration in this population after muscle stretching might reflect the disease progression over time rather than the effect of the muscle stretching intervention. Alternatively, it could be hypothesized that in the context of arterial wall inflammation, which was shown to be increased in PAD patients(48), the mechanical stress on inflamed endothelium due to muscle stretching could potentially increase inflammatory cytokine release such as TNF-α. However, this hypothesis is unlikely since the expression of ICAM of VCAM, of which TNF- α is an upstream regulator in the endothelial inflammation cascade(49), remained unchanged after muscle stretching in PAD patients(38). More research on the topic is needed. In contrast to muscle stretching, a resistance training program with the same duration and frequency in maintenance haemodialysis patients with sarcopenia, who exhibit a comparable elevated inflammation status at baseline, induced the opposite effect. In general, resistance training was shown to reduce levels of TNF-α in healthy older adults (ES up to -0.76, which is larger than the ES observed for stretching in men with PAD and IC (ES=0.64)). The same decrease could be observed after combined training (50). Long-term resistance training was shown to decrease IL-6 in healthy older adults and obese older women. In contrast, no significant changes in IL-6 were observed in healthy older adults and an increase was observed in haemodialysis patients with sarcopenia. Furthermore, long-term combined training also decreased IL-6 levels (50) (Table 4). Overall, these data suggest that a muscle stretching intervention does not affect the protein expression levels of CRP and IL-6 in older participants, regardless of their health status. However, TNF-α levels could be influenced by muscle stretching in participants with an increased inflammatory status at baseline, albeit with a smaller ES compared to resistance or combined exercise.

Stretching can be a potential active control group for resistance exercise regarding antioxidant markers, such as CAT and SOD. Andrade-Lima et al. (38) was the only group reporting on the effects of muscle stretching on mediators that can control oxidative stress. The authors showed a decrease in circulating but not in muscle CAT, an enzyme mitigating oxidative stress by decomposing hydrogen peroxide to water and oxygen (51), in men with PAD and IC symptoms after 12-weeks of muscle stretching exercises twice a week. Resistance exercise in a healthy population for 12 weeks at a ratio of three sessions a week was shown to increase in circulating CAT (no ES reported) (50). Regarding the effect of exercise on muscle CAT, Samjoo et al. (52) showed no effect of endurance training on muscle CAT in sedentary obese men. Andrade-Lima et al. (38) also showed no effect of stretching on circulating or muscle SOD. SOD has important antioxidant properties since it mediates the production of H2O2 from superoxide anion, which can be further catalyzed to water and oxygen by catalase (53). In comparison, Bautmans et al. (50) reported that in healthy older adults, resistance exercise could decrease circulating SOD after 12 weeks consisting of three sessions a week, while aerobic exercise was shown to increase circulating SOD with a medium effect (20). The differences between the directions of effects after aerobic and resistance exercise suggests that the type and intensity of exercise programs are crucial determinants for the outcome of exercise on oxidative stress (54). Regarding muscle SOD, a recent review shows that endurance exercise can decrease SOD in skeletal muscle of obese sedentary men and that lifelong exercise can increase muscle antioxidant enzymes (55).

Our review shows that the effects reported for muscle stretching on oxidative stress markers might be influenced by the health state of participants. However, stretching exercise can be used as an active control group in healthy older adults to control for confounding effects such as social environment. In participants with specific disease characteristics, caution is needed due to the limited extrapolation of the results. While Andrade-Lima et al. (38) and Serra et al. (36) observed no effects on oxidative markers MDA, ox-LDL, Nitrotyrosine, protein carbonyls, and HNE after muscle stretching, Kato et al. (44) in contrast, showed a decrease of oxidative stress, as measured by ROS and MDA-LDL after four weeks of daily muscle stretching for 20 minutes in participants with chronic heart failure. In addition, Kato et al. (44) showed a decrease in MDA-LDL, an oxidized form of LDL after muscle stretching. These effects of muscle stretching were similar to effects observed after exercise, as a 12 week intervention (three session per week lasting 120 minutes each) of Nordic walking in older adults, or 6 months of bicycle ergometer and walking for at least twice a week in cardiac rehabilitation, also showed a decrease on MDA-LDL level (56, 57). No effects were observed in MDA and HNE levels after muscle stretching (36). However, MDA levels decreased after both aerobic exercise and resistance exercise (20, 58). The use of stretching as an active control for exercise effects on HNE remains unclear.

Andrade-Lima et al. (38) showed no effects on NO in men with PAD and IC symptoms after stretching exercises, while LPO levels in both blood and muscle tissue were increased. The review of Ye et al. (20) showed increased levels of NO in older adults after aerobic exercise, as well as reduced levels of circulating oxidant marker LPO. LPO, a marker for oxidative stress related to lipid peroxidation, was increased after muscle stretching in men with PAD and IC symptoms. This increase can be related to the disease onset and progression (38, 45). The increase of NO after exercise was confirmed by the review of Arefirad et al. (59), which also indicated higher levels of NO after aerobic exercise compared to a control group for healthy older adults, obese older adults, and older adults with diabetes.

No changes were observed in ox-LDL, protein carbonyls, and nitrotyrosine after muscle stretching in chronic stroke survivors (36). For ox-LDL, an oxidant marker, no differences were observed after aerobic exercise (20). The oxidative changes in LDL modify both protein and lipid components in a complex process, resulting in the formation of ox-LDL. Ox-LDL contributes to inflammation and is related to the development and progression of atherosclerosis (60). Protein carbonyls are used as marker for protein damage by ROS (36). Radak et al. (61), showed increased serum nitrotyrosine and protein carbonyls levels after an extreme exhaustive aerobic exercise training program (4-day super marathon).

The cell adhesion molecules VCAM-1 and ICAM-1 in the circulation were unchanged after muscle stretching as shown by Andrade-Lima et al. (38) in men with PAD and IC symptoms. Both VCAM and ICAM glycoproteins play a role in the influx of leucocytes to the tissue via adhesion and transmigration through the endothelium, and they are associated with inflammation (62). Koh et al. (63) showed that resistance exercise also did not affect these cell adhesions molecules. In contrast, low-to-moderate intensity aerobic exercise was able to lower cell adhesion molecule levels, while high intensity aerobic exercise was only associated with an acute peak of these molecules, before returning to pre-exercise levels (38, 63).

Kato et al. (44) showed no effects in PTX3 marker and a decrease in fibrinogen after an intensive muscle stretching exercise (seven times weekly for four weeks) in patients with chronic heart failure (ES=-0.6). PTX3 was measured as a vascular inflammatory marker and fibrinogen is a blood clotting factor related to inflammatory processes and can be associated with cardiovascular diseases (44). Zempo-Miyaki et al. (64) observed increased PTX3 levels after an aerobic exercise intervention of eight weeks in older adults. The review of Lin et al. (65) showed a reduction in fibrinogen levels after vigorous exercise intervention in both diabetes and sedentary healthy older adults. Moreover, the review of Thompson et al. (66) observed also lower fibrinogen levels after cardiorespiratory aerobic exercise at both vigorous and moderate levels. At the moment, there is thus insufficient evidence for the use of stretching as a control for the effects of exercise on these markers of vascular inflammation, especially in populations with chronic disease.

Iyalomhe et al. (41) stated that no gene expression changes were observed for the investigated training-induced gene expression profile. On the contrary, Andrade-Lima et al. (38) and Liberman et al. (40) noticed changes in expression of several inflammatory related genes after muscle stretching. Andrade-Lima et al. (38) investigated mRNA levels of inflammatory biomarkers in muscle tissue samples, in contrast to Iyalomhe et al. (41) and Liberman et al. (40) who investigated mRNA levels

in blood, respectively buffy coat samples and in PBMCs. At first sight, this may indicate that muscle stretching exercise might alter the RNA expression of inflammation related genes in the blood circulation, but less in the muscle itself. The expression of pro- and anti-inflammatory genes was less pronounced in the flexibility group compared to the strength training (40) and aerobic training (41). Iyalomhe et al. (41) performed a pathway analysis on both inflammation and cancer related pathways and focused on the interaction within these pathways, in contrast to Liberman et al. (40) who excluded cancer-related pathways. This might explain the discrepancies in gene expression between Iyalomhe et al. (41) and Liberman et al. (40). More research on the effects of muscle stretching on inflammatory related gene expression is needed, since most assessed genes did not overlap, or contradicting results were reported (e.g. IL-6) within the included studies. Moreover, a sedentary control group was not present.

Lastly, muscle stretching had no effect on absolute cell count and percentage of different T-cell subsets in contrast to a resistance endurance training where a decrease in percentages and absolute counts was found after a six week program (42, 43). This suggests that muscle stretching is an appropriate control for studies investigating the effects of exercise on T-cell subsets.

In this systematic literature search we retrieved nine RCTs measuring the effect of muscle stretching exercise programs on the inflammatory profile in older persons. We found no major effects of muscle stretching exercise on the inflammatory status of (healthy) older adults. Most studies reported that muscle stretching has no effect on inflammatory markers and markers related to oxidative stress in older adults. Moreover, muscle stretching exercises had no effect on outcomes related to absolute cell count and percentage of T-cell subsets, except for populations with chronic diseases. Overall, the pro- and anti-inflammatory gene expression was less pronounced in the flexibility group compared to resistance training and aerobic training. However, inconclusive results were found for the effects on oxidative stress markers and gene expression profiles of inflammatory cells. Nonetheless, more dedicated research should be conducted within this area to confirm these findings and clarify the contradictory results. Overall, our systematic review provides evidence for the use of stretching as a suitable control group for exercise immunology studies in older adults. However, caution is warranted in populations with peripheral artery disease.

This systematic review has both strengths and limitations. The novelty of the present systematic review lies in the evaluation of the effects of stretching exercise on the immune system (17, 50, 67). The inclusion of RCTs is a major strength, however, only nine RCTs were identified. Of those, the quality assessment was of some concern in three articles. No meta-analysis was performed because of the heterogeneity regarding the study population (healthy vs disease), duration of intervention, outcome (cell count, gene expression, circulatory markers), and time of assessment. However, ES-values of the muscle stretching interventions were calculated where possible and compared to ES after other types of physical exercise. When the exact type, number and duration of the exercises were unclear, we contacted the authors, but unfortunately, not all authors responded to our inquiries. Only passive (n=4) and active (n=1) muscle stretching interventions were described in the nine articles included in this systematic review, with four studies not defining the type of muscle stretching used. Although this systematic review included 9 studies of which the majority (2/3) were of high quality, there are still some aspects that need investigation in more high qualitative intervention studies focusing on other types of stretching, dose-response aspects and differences according to presence of specific conditions.

CONCLUSION

This systematic review showed no major effects of passive and active muscle stretching exercise on the inflammatory status of older adults and therefore, suggest that a muscle stretching program is a suitable active control group for aerobic and resistance exercise programs. Nevertheless, it is important to take caution when considering populations with peripheral artery disease, as an exacerbation of their inflammatory profile could occur; although it remains unclear whether this exacerbation is due to the passive stretching intervention per se or rather reflecting the overall disease progression.

FUNDING

This systematic review was partly funded by the Wetenschappelijk Fonds Willy Gepts of the Universitair ziekenhuis Brussel (grant numbers WFWG20-02 and WFWG17-02), by the Strategic Growth Funding by the Research Council of the Vrije Universiteit Brussel (grant number SRP59), and by the Fonds Wetenschappelijk Onderzoek Vlaanderen (FWO, Belgium, Grant numbers G0A6W25N, G074221N and 12W2219N).

Conflicts of Interest:
The authors declare that they have no conflicts of interest relevant to the content of this review.

Ethical Approval: Not applicable.

Ethics statement: Not applicable.

Data availability statement:
All data analyzed during this literature study are included in this article. Further inquiries can be directed to the corresponding author.

Author contribution:
EM and LS contributed to the literature search, data extraction, data analysis, and writing of the manuscript. AG contributed the literature search, data extraction, data analysis, and writing of the manuscript. IK, RN and JG reviewed the manuscript. IB designed the study, contributed to the data analysis and interpretation, and writing of the manuscript.

Supplementary materials

Supplement 1: Search key PUBMED:

((exercise, muscle stretching[MeSH Terms]) OR (range of motion, articular[MeSH Terms]) OR (muscle stretching exercise) OR (articular range of motion) OR ("flexibility" AND "exercise") OR ("stretching" AND "exercise") OR (stretching) OR (static stretching) OR ("flexibility" AND "exercise" OR "flexibility" AND "training") OR ("joint" AND "flexibility") OR ("PNF" AND "stretching") OR (proprioceptive neuromuscular facilitation stretching) OR (passive stretching) OR (relaxed stretching) OR (static-passive stretching) OR (isometric stretching) OR (active stretching) OR (static-active stretching) OR (ballistic stretching) OR (dynamic stretching)) AND ((Aged[MeSH Terms]) OR (aged) OR (older adults) OR (older person) OR (older people) OR (elderly)) AND ((inflammation[MeSH Terms]) OR (cytokines[MeSH Terms]) OR (immunity, cellular[MeSH Terms]) OR (inflammation) OR ("inflammatory" AND "profile") OR (inflammatory) OR (cellular immunity) OR ("immune" AND "response") OR ("immune" AND "reaction") OR (cytokines) OR (chemokine) OR ("tumor" AND "necrosis" AND "factor") OR ("C-reactive protein"))

Hits: 1468

Supplement 2: Search key Web of Science:

TS=((muscle stretching exercise) OR (articular range of motion) OR ("flexibility" AND "exercise") OR ("stretching" AND "exercise") OR (stretching) OR (static stretching) OR ("flexibility" AND "exercise" OR "flexibility" AND "training") OR ("joint" AND "flexibility") OR ("PNF" AND "stretching") OR (proprioceptive neuromuscular facilitation stretching) OR (passive stretching) OR (relaxed stretching) OR (static-passive stretching) OR (isometric stretching) OR (active stretching) OR (static-active stretching) OR (ballistic stretching) OR (dynamic stretching)) AND TS=((aged) OR (older adults) OR (older person) OR (older people) OR (elderly)) AND TS=((inflammation) OR ("inflammatory" AND "profile") OR (inflammatory) OR (cellular immunity) OR ("immune" AND "response") OR ("immune" AND "reaction") OR (cytokines) OR (chemokine) OR ("tumor" AND "necrosis" AND "factor") OR ("C-reactive protein"))

Hits: 418

REFERENCES

1. Cosco TD, Howse K, Brayne C. Healthy ageing, resilience and wellbeing. Epidemiol Psychiatr Sci. 26. England2017. p. 579-83.
2. Partridge L, Deelen J, Slagboom PE. Facing up to the global challenges of ageing. Nature. 2018;561(7721):45-56.
3. Strasser B, Wolters M, Weyh C, Krüger K, Ticinesi A. The Effects of Lifestyle and Diet on Gut Microbiota Composition, Inflammation and Muscle Performance in Our Aging Society. Nutrients. 2021;13(6).
4. Sadjapong U, Yodkeeree S, Sungkarat S, Siviroj P. Multicomponent Exercise Program Reduces Frailty and Inflammatory Biomarkers and Improves Physical Performance in Community-Dwelling Older Adults: A Randomized Controlled Trial. Int J Environ Res Public Health. 2020;17(11).
5. Teissier T, Boulanger E, Cox LS. Interconnections between Inflammageing and Immunosenescence during Ageing. Cells. 2022;11(3).
6. Ferrucci L, Fabbri E. Inflammageing: chronic inflammation in ageing, cardiovascular disease, and frailty. Nature Reviews Cardiology. 2018;15(9):505-22.
7. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci. 2000;908:244-54.
8. Lian J, Yue Y, Yu W, Zhang Y. Immunosenescence: a key player in cancer development. J Hematol Oncol. 2020;13(1):151.
9. Aw D, Silva AB, Palmer DB. Immunosenescence: emerging challenges for an ageing population. Immunology. 2007;120(4):435-46.
10. Chen Y, Liu S, Leng SX. Chronic Low-grade Inflammatory Phenotype (CLIP) and Senescent Immune Dysregulation. Clinical Therapeutics. 2019;41(3):400-9.
11. Krabbe KS, Pedersen M, Bruunsgaard H. Inflammatory mediators in the elderly. Experimental Gerontology. 2004;39(5):687-99.
12. Alves AS, Bueno V. Immunosenescence: participation of T lymphocytes and myeloid-derived suppressor cells in aging-related immune response changes. Einstein (Sao Paulo). 2019;17(2):eRB4733.
13. Goronzy JJ, Weyand CM. Understanding immunosenescence to improve responses to vaccines. Nat Immunol. 2013;14(5):428-36.
14. Wang L, Liang Q, Ren Y, Guo C, Ge X, Cheng Q, et al. Immunosenescence: molecular mechanisms and diseases. Signal Transduct Target Ther. 2023;8(1):200.
15. Maldonado E, Morales-Pison S, Urbina F, Solari A. Aging Hallmarks and the Role of Oxidative Stress. Antioxidants (Basel). 2023;12(3).
16. Luo J, Mills K, le Cessie S, Noordam R, van Heemst D. Ageing, age-related diseases and oxidative stress: What to do next? Ageing Res Rev. 2020;57:100982.
17. Mathot E, Liberman K, Cao Dinh H, Njemini R, Bautmans I. Systematic review on the effects of physical exercise on cellular immunosenescence-related markers - An update. Exp Gerontol. 2021;149:111318.
18. Cao Dinh H, Beyer I, Mets T, Onyema OO, Njemini R, Renmans W, et al. Effects of Physical Exercise on Markers of Cellular Immunosenescence: A Systematic Review. Calcified tissue international. 2017;100(2):193-215.
19. El Assar M, Álvarez-Bustos A, Sosa P, Angulo J, Rodríguez-Mañas L. Effect of Physical Activity/Exercise on Oxidative Stress and Inflammation in Muscle and Vascular Aging. Int J Mol Sci. 2022;23(15).

20. Ye Y, Lin H, Wan M, Qiu P, Xia R, He J, et al. The Effects of Aerobic Exercise on Oxidative Stress in Older Adults: A Systematic Review and Meta-Analysis. Front Physiol. 2021;12:701151.
21. Donovan T, Bain AL, Tu W, Pyne DB, Rao S. Influence of Exercise on Exhausted and Senescent T Cells: A Systematic Review. Front Physiol. 2021;12:668327.
22. Turner JE, Brum PC. Does Regular Exercise Counter T Cell Immunosenescence Reducing the Risk of Developing Cancer and Promoting Successful Treatment of Malignancies? Oxid Med Cell Longev. 2017;2017:4234765.
23. Hargreaves M, Spriet LL. Skeletal muscle energy metabolism during exercise. Nat Metab. 2020;2(9):817-28.
24. Chow LS, Gerszten RE, Taylor JM, Pedersen BK, van Praag H, Trappe S, et al. Exerkines in health, resilience and disease. Nat Rev Endocrinol. 2022;18(5):273-89.
25. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol Rev. 2008;88(4):1379-406.
26. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol (1985). 2005;98(4):1154-62.
27. Sharif K, Watad A, Bragazzi NL, Lichtbroun M, Amital H, Shoenfeld Y. Physical activity and autoimmune diseases: Get moving and manage the disease. Autoimmun Rev. 2018;17(1):53-72.
28. Liopeta K, Boubali S, Virgilio L, Thyphronitis G, Mavrothalassitis G, Dimitracopoulos G, et al. cAMP regulates IL-10 production by normal human T lymphocytes at multiple levels: a potential role for MEF2. Mol Immunol. 2009;46(3):345-54.
29. 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(9):607-15.
30. Muscle Stretching Exercises. MeSH Pubmed. 2007.
31. Tock WL, Maheu C, Johnson NA. Considerations of Control Conditions Designs in Randomized Controlled Trials of Exercise Interventions for Cancer Survivors. Canadian Journal of Nursing Research. 2022;54(4):377-91.
32. Waters L, Reeves M, Fjeldsoe B, Eakin E. Control Group Improvements in Physical Activity Intervention Trials and Possible Explanatory Factors: A Systematic Review. Journal of Physical Activity and Health. 2012;9(6):884-95.
33. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan—a web and mobile app for systematic reviews. Systematic Reviews. 2016;5(1):210.
34. Rohatgi A. WebPlotDigitizer 2023 [4.6:[Available from: https://automeris.io/WebPlotDigitizer.html.
35. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ. 2019;366:l4898.
36. Serra MC, Hafer-Macko CE, Robbins R, O'Connor JC, Ryan AS. Randomization to Treadmill Training Improves Physical and Metabolic Health in Association With Declines in Oxidative Stress in Stroke. Arch Phys Med Rehabil. 2022;103(11):2077-84.
37. de Paulo TRS, Winters-Stone KM, Viezel J, Rossi FE, Simoes RR, Tosello G, et al. Effects of resistance plus aerobic training on body composition and metabolic markers in older breast cancer survivors undergoing aromatase inhibitor therapy. Exp Gerontol. 2018;111:210-7.
38. Andrade-Lima A, Silva Junior N, Chehuen M, Miyasato R, Souza RWA, Leicht AS, et al. Walking Training Improves Systemic and Local Pathophysiological Processes in Intermittent Claudication. Eur J Vasc Endovasc Surg. 2021;61(6):954-63.
39. Rodrigues-Krause J, Farinha JB, Ramis TR, Macedo RCO, Boeno FP, Dos Santos GC, et al. Effects of dancing compared to walking on cardiovascular risk and functional capacity of older women: A randomized controlled trial. Exp Gerontol. 2018;114:67-77.
40. Liberman K, Njemini R, Forti LN, Cools W, Debacq-Chainiaux F, Kooijman R, et al. Three Months of Strength Training Changes the Gene Expression of Inflammation-Related Genes in PBMC of Older Women: A Randomized Controlled Trial. Cells. 2022;11(3).
41. Iyalomhe O, Chen Y, Allard J, Ntekim O, Johnson S, Bond V, et al. A standardized randomized 6-month aerobic exercise-training down-regulated pro-inflammatory genes, but up-regulated anti-inflammatory, neuron survival and axon growth-related genes. Exp Gerontol. 2015;69:159-69.
42. Cao Dinh H, Bautmans I, Beyer I, Onyema OO, Liberman K, De Dobbeleer L, et al. Six weeks of strength endurance training decreases circulating senescence-prone T-lymphocytes in cytomegalovirus seropositive but not seronegative older women. Immun Ageing. 2019;16:17.
43. Cao Dinh H, Njemini R, Onyema OO, Beyer I, Liberman K, De Dobbeleer L, et al. Strength endurance training but not intensive strength training reduces senescence-prone T-cells in peripheral blood in community-dwelling elderly women. J Gerontol A Biol Sci Med Sci. 2018.
44. Kato M, Masuda T, Ogano M, Hotta K, Takagi H, Tanaka S, et al. Stretching Exercises Improve Vascular Endothelial Dysfunction Through Attenuation of Oxidative Stress in Chronic Heart Failure Patients With an Implantable Cardioverter Defibrillator. J Cardiopulm Rehabil Prev. 2017;37(2):130-8.
45. Signorelli SS, Scuto S, Marino E, Xourafa A, Gaudio A. Oxidative Stress in Peripheral Arterial Disease (PAD) Mechanism and Biomarkers. Antioxidants (Basel). 2019;8(9).
46. Signorelli SS, Mazzarino MC, Di Pino L, Malaponte G, Porto C, Pennisi G, et al. High circulating levels of cytokines (IL-6 and TNFalpha), adhesion molecules (VCAM-1 and ICAM-1) and selectins in patients with peripheral arterial disease at rest and after a treadmill test. Vasc Med. 2003;8(1):15-9.
47. Murabito JM, Keyes MJ, Guo C-Y, Keaney JF, Jr., Vasan RS, D'Agostino RB, Sr., et al. Cross-sectional relations of multiple inflammatory biomarkers to peripheral arterial disease: The Framingham Offspring Study. Atherosclerosis. 2009;203(2):509-14.
48. Bernelot Moens SJ, Stoekenbroek RM, van der Valk FM, Verweij SL, Koelemay MJW, Verberne HJ, et al. Carotid arterial wall inflammation in peripheral artery disease is augmented by type 2 diabetes: a cross-sectional study. BMC Cardiovascular Disorders. 2016;16(1):237.
49. Pober JS, Sessa WC. Inflammation and the blood microvascular system. Cold Spring Harb Perspect Biol. 2014;7(1):a016345.
50. Bautmans I, Salimans L, Njemini R, Beyer I, Lieten S, Liberman K. The effects of exercise interventions on the inflammatory profile of older adults: A systematic review of the recent literature. Exp Gerontol. 2021;146:111236.
51. Gebicka L, Krych-Madej J. The role of catalases in the prevention/promotion of oxidative stress. J Inorg Biochem. 2019;197:110699.
52. Samjoo IA, Safdar A, Hamadeh MJ, Raha S, Tarnopolsky MA. The effect of endurance exercise on both skeletal muscle and systemic oxidative stress in previously sedentary obese men. Nutr Diabetes. 2013;3(9):e88.
53. Fukai T, Ushio-Fukai M. Superoxide dismutases: role in redox signaling, vascular function, and diseases. Antioxid Redox Signal. 2011;15(6):1583-606.

54. Thirupathi A, Wang M, Lin JK, Fekete G, István B, Baker JS, et al. Effect of Different Exercise Modalities on Oxidative Stress: A Systematic Review. Biomed Res Int. 2021;2021:1947928.
55. Powers SK, Goldstein E, Schrager M, Ji LL. Exercise Training and Skeletal Muscle Antioxidant Enzymes: An Update. Antioxidants (Basel). 2022;12(1).
56. Takashima A, Ise T, Yagi S, Iwase T, Kimura S, Ueda Y, et al. Cardiac rehabilitation reduces serum levels of oxidized low-density lipoprotein. Circ J. 2014;78(11):2682-7.
57. Kawamoto R, Kohara K, Katoh T, Kusunoki T, Ohtsuka N, Abe M, et al. Changes in oxidized low-density lipoprotein cholesterol are associated with changes in handgrip strength in Japanese community-dwelling persons. Endocrine. 2015;48(3):871-7.
58. Wang Y, Luo D, Jiang H, Song Y, Wang Z, Shao L, et al. Effects of physical exercise on biomarkers of oxidative stress in healthy subjects: A meta-analysis of randomized controlled trials. Open Life Sciences. 2023;18(1).
59. Arefirad T, Seif E, Sepidarkish M, Mohammadian Khonsari N, Mousavifar SA, Yazdani S, et al. Effect of exercise training on nitric oxide and nitrate/nitrite (NOx) production: A systematic review and meta-analysis. Front Physiol. 2022;13:953912.
60. Birukov KG. Oxidized lipids: the two faces of vascular inflammation. Curr Atheroscler Rep. 2006;8(3):223-31.
61. Radák Z, Ogonovszky H, Dubecz J, Pavlik G, Sasvari M, Pucsok J, et al. Super□marathon race increases serum and urinary nitrotyrosine and carbonyl levels. European journal of clinical investigation. 2003;33(8):726-30.
62. Kaur R, Singh V, Kumari P, Singh R, Chopra H, Emran TB. Novel insights on the role of VCAM-1 and ICAM-1: Potential biomarkers for cardiovascular diseases. Ann Med Surg (Lond). 2022;84:104802.
63. Koh Y, Park J. Cell adhesion molecules and exercise. J Inflamm Res. 2018;11:297-306.
64. Zempo-Miyaki A, Fujie S, Sato K, Hasegawa N, Sanada K, Maeda S, et al. Elevated pentraxin 3 level at the early stage of exercise training is associated with reduction of arterial stiffness in middle-aged and older adults. J Hum Hypertens. 2016;30(9):521-6.
65. Lin X, Zhang X, Guo J, Roberts CK, McKenzie S, Wu WC, et al. Effects of Exercise Training on Cardiorespiratory Fitness and Biomarkers of Cardiometabolic Health: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. J Am Heart Assoc. 2015;4(7).
66. Thompson G, Davison GW, Crawford J, Hughes CM. Exercise and inflammation in coronary artery disease: A systematic review and meta-analysis of randomised trials. J Sports Sci. 2020;38(7):814-26.
67. Salimans L, Liberman K, Njemini R, Kortekaas Krohn I, Gutermuth J, Bautmans I. The effect of resistance exercise on the immune cell function in humans: A systematic review. Exp Gerontol. 2022;164:111822.

Less Inflammatory Debris, Improved Immunity from Immune Detox: A New Perspective on the Benefits of Exercise in Chronic Disease

Asghar Abbasi (PhD), William Stringer (MD)

[1] Respiratory Research Center, The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, 1124 W Carson St., Torrance, CA 90502
[2] Division of Respiratory and Critical Care Physiology and Medicine, Harbor-UCLA Medical Center, 1000 W Carson St., Torrance, CA 90509

ABSTRACT

The immunological benefits of exercise are commonly attributed to its immune-boosting effects such as the release of exercise-induced factors (e.g., exerkines) and activation of anti-inflammatory molecules. However, this may not fully explain its benefits in chronic inflammatory conditions. We propose a complementary view whereby exercise potentially functions as a biological detoxifier by removing harmful immunological debris such as damage-associated molecular patterns (DAMPs), senescent cells, dysfunctional mitochondria and pro-inflammatory extracellular vesicles (EVs) that drive chronic immune activation. We highlight key mechanisms by which exercise may reduce or remove these harmful signals, including autophagy and mitophagy activation, enhanced efferocytosis, reduced senescence burden, and modulation of EV cargo. This "immune detox" model may help explain the clinical benefits of exercise in conditions where the immune system is overactivated, not deficient. It shifts the narrative from immune boosting to restoring immune balance, and could have potentially important implications for biomarker discovery and personalized exercise prescriptions in chronic disease.

**Corresponding author:*
Asghar Abbasi, PhD
The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center
Division of Respiratory & Critical Care Physiology & Medicine
1124 W Carson St, CDCRC Building
Torrance, CA 90502
Email: asghar.abbasi@lundquist.org
Tel:+1-(310)-974-9533

INTRODUCTION

Shifting the Paradigm

The immunological benefits of exercise are widely recognized to enhance the production of beneficial signaling molecules. From the secretion of myokines such as IL-6, IL-15, and irisin, to systemic changes in catecholamines and lactate, the dominant view has been that physical activity stimulates a cascade of molecules that support immune surveillance, repair, and immune downregulation (1). This framework has been particularly influential in the field of exercise immunology, leading to a continued focus on identifying and quantifying exercise-induced biomarkers associated with anti-inflammatory or immunomodulatory effects. However, this model may be incomplete, especially in the context of chronic diseases characterized by persistent inflammation and immune dysregulation. In these persistent conditions, such as chronic obstructive pulmonary disease (COPD), asthma, idiopathic pulmonary fibrosis (IPF) (2-6), acute lung and liver disease (7-9), diabetes mellitus (10, 11), cancer (12), Alzheimer's disease (13), autoimmune diseases (14), and aging (15, 16), immune dysfunction appears to be less about a lack of immune activation and more about the persistence of harmful signals. Damaged and senescent cells accumulate in these chronic states, releasing a spectrum of pro-inflammatory and danger-associated molecules that chronically stimulate the immune system (6, 17). The result is not immune deficiency, but an immunologically overactive system that struggles to maintain homeostasis in the face of continuous inflammatory stimuli from internal sources. In fact, an editorial by Dr Esch et al. (2020) highlights the widespread clinical use of monoclonal antibody therapies specifically designed to neutralize unregulated secretion of circulating proinflammatory cytokines for the treatment of diverse autoimmune syndromes (18). These therapeutic approaches highlight the clinical relevance of persistent inflammatory signals.

In this perspective article, we hypothesize that regular physical exercise contributes to immune rebalancing, not only by inducing beneficial exercise-induced factors, but also by reducing or removing pro-inflammatory cellular byproducts such as DAMPs, senescent signals, dysfunctional mitochondria, and inflammatory EVs, that we collectively

refer to as "inflammatory debris" or "immune clutter". This concept which we describe as "immune detox" emphasizes a "*subtractive model*" of immune regulation, where exercise promotes immune homeostasis by removing internal drivers of chronic inflammation. These terms are used metaphorically throughout the manuscript to describe biological processes that reduce harmful immunological molecules and restore balance.

The Immunological Burden on Chronic Disease

Chronic diseases are increasingly recognized as states of sustained immunological stress, driven not only by pathogens or external insults, but by the persistent accumulation of endogenous danger signals. DAMPs are released or exposed during a variety of cellular processes, including injury, necrosis, mitochondrial dysfunction, or senescence. Rather than resolving over time, DAMPs remain elevated in many chronic conditions including COPD, obesity, type 2 diabetes, autoimmune diseases and aging, resulting in prolonged immune activation and systemic inflammation. The most well described and accepted DAMPs are nucleic acids such as mitochondrial DNA (mtDNA) (13, 19-21), HMGB1 (high mobility group box 1) (18, 22), histones (23), and S100 proteins (24). Other nuclear or cytosolic components, when misplaced or mispackaged, also signal tissue damage. Given the similarities between DAMPs and pathogen-associated molecular patterns (PAMPs), it should be no surprise that DAMPs are sensed by innate immune receptors such as toll-like receptors (TLRs) and NOD-like receptors (NLRs), initiating inflammatory cascades that perpetuate low-grade but chronic immune stimulation (25-29).

Senescent cells also contribute to this inflammatory load by releasing a complex mix of pro-inflammatory factors, collectively known as senescence-associated secretory phenotype (SASP)(30), which includes cytokines (e.g., IL-6, IL-8), chemokines (e.g., CXCL1, CCL2), matrix remodeling enzymes (e.g., MMPs), and growth factors. Their persistence in tissues adds another layer to immune dysregulation, especially in the aging population (6, 11, 16). One or more SASP components likely mediate chronic inflammation and affect the growth and survival of tumor cells (30, 31). Its regulation occurs at multiple levels, including chromatin remodeling and activation of transcription factors such as C/EBP and NF-κB. Nevertheless, SASP components can also support tissue repair, immune clearance, and wound healing, making them promising targets for therapeutic modulation (31, 32).

Mitochondria are bacterial endosymbionts that have evolved to be vital organelles in cellular energy production. Despite this, mitochondria are frequent sources of immune-activating signals when damaged (27). Mitochondrial DNA (mtDNA), which has a bacterial-like, double-stranded circular structure, can be released into the cytosol or extracellular space under cellular stress such as ATP or LPS stimulation, where it acts as a potent DAMP that activates the innate immune system via receptors like TLR4 or TLR9 (20, 33). Among the best-characterized mitochondrial genes that trigger immune responses are *MT-ND1*, *MT-ND2*, MT-ND4 and MT-CO1 (34). Human neutrophils exposed to purified mtDNA released matrix metallopeptidases MMP-8 and MMP-9 (35), and incubation of human embryonic kidney cells with serum from acute myocardial infarction patients contains elevated levels of mtDNA induced TLR9-dependent NF-κB activity (36). Together, these findings highlight the potent immunostimulatory capacity of mitochondrial components, particularly mtDNA, and underscore their emerging role as key inflammatory mediators.

A less often discussed, but increasingly important component of this immunological milieu is EVs. Long considered vehicles of intercellular communication, EVs also serve as a mechanism for exporting unwanted or harmful cellular contents in a protected fashion. EVs often carry inflammatory cargo in chronic diseases, including damaged nucleic acids, oxidized lipids, and dysfunctional mitochondrial proteins (37). Emerging evidence suggests that these vesicles are not merely messengers but active participants in immune signaling, especially when their cargo consists of DAMP-like molecules. Several studies have demonstrated that EVs mediate inflammatory responses by delivering mtDNA (37). mtDNA levels are elevated in EVs and the release of mtDNA into EVs is suggested to coincide with an increase in the expression of markers associated with DNA damage, proinflammatory cytokines and senescence (27, 38, 39). Besides mtDNA, mitochondrial RNAs (e.g., tRNAs and rRNAs), which are recognized as key DAMPs and involved in triggering inflammation, are also carried by EVs (40, 41). Like mtDNA, mitochondrial RNAs (mtRNAs) can originate from mitochondrial fragments of stressed or damaged cells and once delivered to target cells via EVs, they can activate TLRs and trigger inflammatory pathways (42, 43). Hough and colleagues (2018) demonstrated that EVs can transfer mitochondria to target T cells and integrate with their mitochondrial network, leading to increased production of reactive oxygen species (ROS) and activation of inflammatory signaling pathways within the T cells (44). These findings suggest that the mtRNA and mtDNA content within EVs may hold clinical relevance and could serve as potential diagnostic and prognostic biomarkers for chronic inflammatory diseases.

Thus, the immune system in chronic disease settings appears to be burdened not only by defective regulation, but also by the sheer volume of inflammatory signals that sustain its activation. Addressing this "inflammatory debris" may be a more effective strategy for restoring immune balance than further stimulating a system that is already overwhelmed.

Exercise as an Immune Detoxifier

Exercise involves a diverse set of molecular and cellular pathways that enhance tissue renewal, metabolic homeostasis, and immuno-regulation. Importantly, many of these pathways directly contribute to the clearance of harmful cellular byproducts. In contrast to the conventional view of exercise as a stimulator of beneficial exerkines, an equally powerful role may lie in the potential for exercise to enhance clearance mechanisms, acting as a biological detoxifier.

A cornerstone of this detoxifying effect is the activation of autophagy and mitophagy, as shown by He et al. (2012) and Laker et al. (2017), where exercise upregulated muscle autophagy and mitochondrial quality control mechanisms, respectively (45, 46). Indeed, exercise-induced autophagy and mitophagy seem to be involved in mediating many of the beneficial effects of exercise. These conserved pathways are responsible for the degradation and recycling of damaged

organelles and proteins. Exercise, particularly aerobic and endurance-based exercise, increases autophagic capacity by upregulation of key regulators such as AMP-activated protein kinase (AMPK), forkhead box O (FOXO), transcription factor EB (TFEB), sirtuin 1 (SIRT1), and peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1α) (47). Exercise-induced AMPK can also suppress mammalian target of rapamycin (mTOR), which is a known suppressor of autophagy (47). The role of exercise-induced autophagy has been investigated by inhibiting autophagy using the lysosomal inhibitor chloroquine, administered 5 days per week for 16 weeks in an animal model (48). This intervention has led to the development of sporadic inclusion body myositis, a condition characterized by muscle weakness and wasting (48). Resistance exercise training prevented chloroquine-induced increases in ATG6 (Beclin-1) and p62 (48), thereby improving autophagy, muscle strength, and preventing myositis.

Through mitophagy, exercise facilitates the removal of dysfunctional mitochondria, thereby reducing the leakage of ROS and mtDNA, which are both potent DAMPs that drive immune activation. This process is mediated by key molecular pathways, particularly the PINK1/Parkin axis (49). Upon mitochondrial damage, PINK1 accumulates on the outer mitochondrial membrane and recruits the E3 ubiquitin ligase Parkin, which ubiquitinates outer membrane proteins and marks the mitochondria for degradation via lysosomal autophagosomes (49). By selectively clearing damaged mitochondria, exercise-induced mitophagy preserves mitochondrial quality and supports cellular homeostasis.

Another critical mechanism is enhanced efferocytosis, the process by which phagocytic cells, particularly macrophages, clear apoptotic cells (e.g., neutrophils) to maintain tissue homeostasis. In chronic diseases such as COPD, asthma, atherosclerosis, lupus, cystic fibrosis, rheumatoid arthritis, neurodegenerative diseases (e.g., Alzheimer's, Parkinson's), type 2 diabetes, obesity, and some cancers, efferocytosis is often impaired, resulting in secondary necrosis and the release of additional pro-inflammatory contents (50-56). While direct evidence regarding the effects of either acute or chronic exercise on macrophage efferocytosis is currently lacking, exercise training has been shown to reprogram macrophages toward a more efficient, anti-inflammatory phenotype, thereby enhancing their ability to resolve inflammation (57). Since timely clearance of apoptotic cells prevents the release of inflammatory DAMPs (58), interventions that reduce DAMPs (such as exercise) may indirectly support and improve apoptotic cell clearance.

Senescence is another target of exercise-mediated immune detox. Studies in aging and metabolic disease models have demonstrated that regular exercise reduces the number of exhausted/senescent cells as well as circulating SASP factors (59, 60). In addition, increased cardiorespiratory fitness can protect against the production/accumulation of senescent cells, including immune cell populations (60, 61). For example, it has been demonstrated that individuals with an above-average cardiorespiratory fitness measured by VO_2max (47.3 ml/kg/min) had 37% fewer KLRG1+/CD57+ and KLRG1+/CD28− senescent T cells than individuals with an average or below-average VO_2max (43.0 and 34.4 ml/kg/min, respectively) (62). Whether reduced senescent cells are due to direct effects on cellular senescence or enhanced immune clearance of senescent cells remains under investigation, but the result is a measurable reduction in pro-inflammatory signaling (63, 64).

A particularly intriguing detoxification pathway involves the modulation of EVs. As previously noted, beyond transporting secreted exerkines and immunoregulatory molecules, EVs can also carry harmful cargo including mtDNA, pro-inflammatory cytokines and chemokines, nuclear debris, and dysfunctional proteins, all of which contribute to immune activation. Exercise has been shown to change not only the abundance of circulating EVs, but also their molecular cargo (65-68). In both animal models and human studies, exercise appears to shift EV profiles away from pro-inflammatory content toward those enriched in regulatory or restorative molecules (69-72). This may reflect a functional reorganization of the EV system, shifting from disseminating inflammatory signals to exporting intracellular waste, depending on physiological context. Recently, we observed a reduction in the EV content of mitochondrially encoded transcripts, including *MT-ND1*, *MT-ND2*, *MT-NDP28*, *MT-ND3* and *MT-RNR1*, at peak exercise compared to rest, following exercise training interventions in two different patient groups (COPD and long COVID) (*Abstract published* (73), and *manuscripts currently under review*). These findings support the idea that exercise facilitates the clearance or suppression of harmful mitochondrial-derived EV cargo, representing a potential mechanism for immune restoration. In addition, exercise-induced EVs may contribute to macrophage efferocytosis. EVs can enhance macrophage efferocytosis by modulating macrophage polarization, thereby improving apoptotic cell clearance and promoting inflammation resolution (74, 75). Specifically, EVs may increase the expression of efferocytosis receptors such as Tim4 on macrophages, enhancing their capacity to engulf apoptotic cells (74). EVs can also deliver functional molecules like milk fat globule-EGF factor 8 (MFGE8), which further promote efferocytosis (76). Notably, we recently found that C1q proteins are elevated in serum EVs from smoke-exposed mice following 1 hour of aerobic exercise compared to their non-exercised counterparts (*Abstract published* (77)). C1q, a well-known component of the classical complement system, also acts as a pro-efferocytic signal, enhancing the uptake of apoptotic cells while limiting TNFα production by mouse and human macrophages (78-80). The unpublished data from our lab, although preliminary, add empirical support to the proposed detoxification hypothesis, and are the focus of ongoing mechanistic investigations.

Collectively, these mechanisms illustrate how exercise acts as a biological clean-up mechanism (see *Figure 1*), leading to reduced immune activation. Rather than merely introducing beneficial factors, exercise also diminishes the presence of harmful or inflammatory ones. In principle, this could alleviate chronic immune stimulation and allow the system to return to a more responsive and regulated state. We hypothesize that exercise, including acute bouts, reduces the EV content of mitochondrially-encoded RNAs (e.g., *MT-ND1*, *MT-ND2*, *MT-ND3*, *MT-RNR1*, *MT-RNR2*) by enhancing mitochondrial quality control via coordinated activation of AMPK, PGC-1α, FOXO, and SIRT1 signaling pathways. Although direct

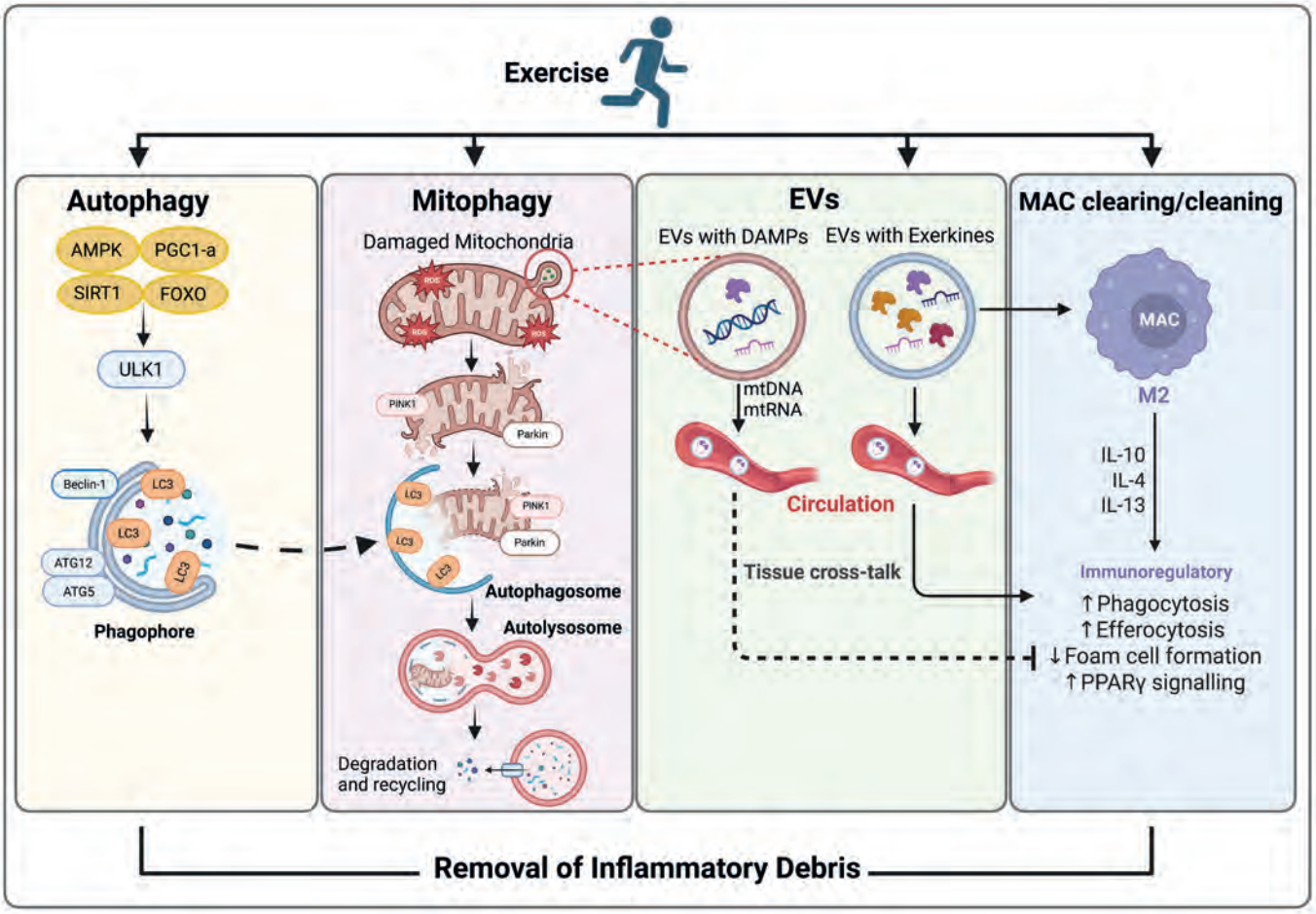


Figure 1. Exercise-induced autophagy, mitophagy, macrophage pro-resolving function and modulation of EV content contribute to cellular detoxification and immune resolution. Exercise activates key signaling molecules such as AMPK, PGC-1α, SIRT1, and FOXO, which initiate the ULK1–Beclin-1–LC3 autophagy machinery. This promotes the formation of autophagosomes and enhances mitophagy, mitochondria-specific autophagy. Dysfunctional mitochondria produce excess reactive oxygen species (ROS) and release mitochondrial DAMPs such as mtDNA and mtRNAs, which may be exported via EVs. Exercise reduces the abundance of EVs and DAMPs, while increasing the production of EVs laden exerkines that influence immune cells. In particular, exercise-induced EVs can promote macrophage polarization toward an M2-like phenotype, enhancing phagocytosis, efferocytosis, and PPARγ signaling, while reducing foam cell formation. Collectively, these pathways support a coordinated detoxification response, contributing to improved mitochondrial quality and immune homeostasis by removing inflammatory debris. EV: Extracellular vesicles; MAC: macrophage; DAMP: danger-associated molecular patterns; ROS: reactive oxygen species.

evidence is currently lacking, it is plausible that exercise-induced activation of signaling molecules such as AMPK, PGC-1α, FOXO, or SIRT1 contributes to the observed reduction in mtRNA and mtDNA content in EVs. This reduction may reflect improved mitochondrial integrity, turnover, or reduced cellular stress, rather than direct pathway-specific modulation. Future studies are needed to dissect these mechanisms.

Reframing the Concept of Exercise-Induced Benefits

If we embrace the view that exercise reduces harmful inflammatory immunological burden rather than simply enhancing beneficial signals, it will require a fundamental shift in how we understand, study, and prescribe physical activity, particularly in the context of chronic disease. The traditional narrative has emphasized the "addition" model, in which exercise increases anti-inflammatory cytokines, mobilizes immune cells, and releases myokines and metabolites that enhance host defense and tissue repair. While these phenomena are well-documented, they do not appear to fully account for the therapeutic effects observed in individuals with chronic inflammatory conditions. In such individuals, the immune system is overactive, bombarded by DAMPs, senescent signaling, and pro-inflammatory EVs. In this context, the value of exercise may lie in its potential to remove the drivers of this chronic activation, essentially dampening the background noise so that productive immune responses can emerge. This subtractive or detoxifying model reframes exercise not as a simple immune stimulant, but as a homeostatic regulator that clears internal debris and restores equilibrium.

This perspective may also help explain why modest doses of exercise, sometimes even below the recommended thresholds, can result in profound clinical improvements in populations with chronic illness or immunosenescence. These populations may not need more immune activation; they need less immune distraction. By reducing the load of pro-inflammatory signals, exercise may restore immune precision and functional reserve. Importantly, this conceptual shift has implications for biomarker development and exercise prescription. Biomarkers of benefit should not be limited to increased levels of known anti-inflammatory mediators but should also include reductions in molecules that reflect immune clutter (e.g., circulating

mtDNA, senescence-associated transcripts, SASP factors, and inflammatory EVs). Similarly, exercise prescriptions could be personalized not only by intensity and volume, but also by the individual's inflammatory burden and capacity to engage in immune restoration.

In sum, this reframing opens the door for a more nuanced, mechanism-informed understanding of exercise in chronic disease. It positions physical activity as a systemic regulator of immune tone, not merely by boosting the system, but rather by clearing the path for it to function effectively.

It is important to recognize that the immune-activating and immune-detoxifying effects of exercise are not mutually exclusive but may function synergistically depending on the physiological context. For instance, during acute infection or immunosuppression, the stimulatory effects of exercise on immune surveillance and effector functions may be advantageous. In contrast, in chronic disease conditions (which are characterized by sustained immune activation and systemic inflammation) the ability of exercise to attenuate immunological burden through clearance of pro-inflammatory mediators may be more therapeutically relevant. This integrated framework acknowledges that exercise exerts both immunostimulatory and immunoregulatory effects, with their relative predominance shaped by factors such as disease type, biological age, physical fitness, and metabolic status.

While the detox model presents a compelling framework, it is important to recognize its potential limitations. Under certain conditions, excessive autophagy or mitophagy may be maladaptive, leading to cellular dysfunction or energy depletion (81-85). Furthermore, in some chronic diseases, clearance pathways such as efferocytosis or autophagy may be too impaired to fully respond to exercise stimuli, limiting the detox potential (11, 20, 51, 86). Finally, if immune activity is overly affected, it could potentially suppress essential immune surveillance. These limitations underscore the need for mechanistic validation and the identification of optimal exercise thresholds that balance immune restoration with necessary immune vigilance.

Future Directions and Therapeutic Potential

The idea that exercise promotes immune health by reducing cellular waste and inflammatory burden presents a number of promising avenues for future research and therapeutic innovation. While preclinical and *in vitro* studies have provided strong foundational evidence, direct clinical validation of these detoxification mechanisms, particularly exercise-induced EV cargo modulation and enhanced efferocytosis, in human populations remains limited. Future studies in animal models and human populations with chronic diseases are needed to confirm whether these mechanisms translate into measurable therapeutic effects.

As this detoxification framework gains traction, several key areas deserve focused investigation. First, the development of biomarkers of immune inflammatory debris or clutter could greatly enhance our ability to assess exercise responsiveness in chronic disease populations. Current research predominantly measures increases in exercise-induced factors, but equally important may be reductions in circulating DAMPs (e.g., HMGB1, mtDNA), senescence-associated transcripts (e.g., p16, IL-6, CXCL1), dysfunctional mitochondria markers, and pro-inflammatory EVs. Standardizing these metrics could help identify individuals most in need of "immune unloading" and track their physiological response to training.

Second, this perspective opens the possibility for therapeutic synergy. Interventions aimed at reducing immune burden such as senolytics, mitophagy enhancers, or anti-inflammatory diets may act synergistically with exercise to accelerate immune restoration. Rather than viewing exercise as a standalone tool, it could be strategically integrated into multi-modal treatment plans that combine physical activity with agents targeting cellular senescence, mitochondrial health, or vesicle biogenesis.

Third, understanding individual variability in "immune detox" capacity will be essential. Some individuals, due to age, disease state, or genetic predisposition, may exhibit impaired efferocytosis (e.g., in COPD), sluggish autophagy, or dysregulated EV release. These individuals might require longer or differently structured training regimens to achieve the same immunological benefits. Investigating the molecular "non-responder" phenotype in this context could be particularly valuable.

Finally, the detox model has clear relevance in emerging areas such as long COVID, immunosenescence, and cancer-related immune exhaustion. These conditions are all marked by a buildup of internal danger signals and impaired immune resolution. Exercise interventions tailored to reduce this load may provide a safe and scalable strategy to restore immune resilience in diverse clinical populations. Together, these directions underscore the potential of a more refined, mechanism-based application of exercise as a therapeutic agent, not just to stimulate immunity, but to improve removal of inflammatory debris.

CONCLUSION

The conventional narrative of exercise as an immune booster has advanced our understanding of how physical exercise supports health. However, in the context of chronic diseases characterized by immune overactivation and persistent inflammation, this perspective may be incomplete. We propose that a major, underappreciated benefit of exercise lies in its ability to reduce harmful cellular byproducts, what we term 'inflammatory debris' or clutter. By promoting autophagy, enhancing efferocytosis, reducing senescence, and reshaping extracellular vesicle cargo, exercise acts as a biological detoxifier that restores immune balance. This framework offers a compelling explanation for the broad clinical benefits of exercise rehabilitation, especially in populations where the immune system is not underperforming, but is overactive. Although promising, this model remains a hypothesis and further clinical validation is essential. Recognizing and measuring this detoxifying role opens the door for new biomarkers, personalized interventions, and therapeutic strategies that position exercise and exercise rehabilitation as a precision tool for immune restoration.

REFERENCES

1. Chow LS, Gerszten RE, Taylor JM, Pedersen BK, van Praag H, Trappe S, et al. Exerkines in health, resilience and disease. Nat Rev Endocrinol. 2022;18(5):273-89.
2. Pouwels SD, Heijink IH, ten Hacken NH, Vandenabeele P, Krysko DV, Nawijn MC, et al. DAMPs activating innate and adaptive immune responses in COPD. Mucosal Immunol. 2014;7(2):215-26.
3. Dong L-L, Liu Z-Y, Chen K-J, Li Z-Y, Zhou J-S, Shen H-H, et al. The persistent inflammation in COPD: is autoimmunity the core mechanism? European Respiratory Review. 2024;33(171):230137.
4. Peebles RS, Jr., Aronica MA. Proinflammatory pathways in the pathogenesis of asthma. Clin Chest Med. 2019;40(1):29-50.
5. Huang X, Tan X, Liang Y, Hou C, Qu D, Li M, et al. Differential DAMP release was observed in the sputum of COPD, asthma and asthma-COPD overlap (ACO) patients. Sci Rep. 2019;9(1):19241.
6. Barnes PJ, Baker J, Donnelly LE. Cellular senescence as a mechanism and target in chronic lung diseases. Am J Respir Crit Care Med. 2019;200(5):556-64.
7. Kuipers MT, van der Poll T, Schultz MJ, Wieland CW. Bench-to-bedside review: Damage-associated molecular patterns in the onset of ventilator-induced lung injury. Critical care. 2011;15:1-11.
8. Marques PE, Amaral SS, Pires DA, Nogueira LL, Soriani FM, Lima BH, et al. Chemokines and mitochondrial products activate neutrophils to amplify organ injury during mouse acute liver failure. Hepatology. 2012;56(5):1971-82.
9. Huidobro C, Martín-Vicente P, López-Martínez C, Alonso-López I, Amado-Rodríguez L, Crespo I, et al. Cellular and molecular features of senescence in acute lung injury. Mech Ageing Dev. 2021;193:111410.
10. Shin JJ, Lee EK, Park TJ, Kim W. Damage-associated molecular patterns and their pathological relevance in diabetes mellitus. Ageing Res Rev. 2015;24(Pt A):66-76.
11. Palmer AK, Tchkonia T, LeBrasseur NK, Chini EN, Xu M, Kirkland JL. Cellular senescence in type 2 diabetes: a therapeutic opportunity. Diabetes. 2015;64(7):2289-98.
12. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med. 2019;25(12):1822-32.
13. Wilkins HM, Carl SM, Greenlief AC, Festoff BW, Swerdlow RH. Bioenergetic dysfunction and inflammation in Alzheimer's disease: a possible connection. Front Aging Neurosci. 2014;6:311.
14. Romerio A, Peri F. Increasing the chemical variety of small-molecule-based TLR4 modulators: an overview. Front Immunol. 2020;11:1210.
15. Kapetanovic R, Bokil NJ, Sweet MJ. Innate immune perturbations, accumulating DAMPs and inflammasome dysregulation: A ticking time bomb in ageing. Ageing Res Rev. 2015;24(Pt A):40-53.
16. van Deursen JM. The role of senescent cells in ageing. Nature. 2014;509(7501):439-46.
17. Ma KC, Schenck EJ, Pabon MA, Choi AMK. The role of danger Signals in the pathogenesis and perpetuation of critical illness. Am J Respir Crit Care Med. 2018;197(3):300-9.
18. Esch T, Stefano GB, Ptacek R, Kream RM. Emerging roles of blood-borne intact and respiring mitochondria as bidirectional mediators of pro- and anti-inflammatory processes. Med Sci Monit. 2020;26:e924337.
19. Simmons JD, Lee YL, Mulekar S, Kuck JL, Brevard SB, Gonzalez RP, et al. Elevated levels of plasma mitochondrial DNA DAMPs are linked to clinical outcome in severely injured human subjects. Ann Surg. 2013;258(4):591-6; discussion 6-8.
20. Nakahira K, Haspel JA, Rathinam VA, Lee SJ, Dolinay T, Lam HC, et al. Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol. 2011;12(3):222-30.
21. Nakahira K, Kyung SY, Rogers AJ, Gazourian L, Youn S, Massaro AF, et al. Circulating mitochondrial DNA in patients in the ICU as a marker of mortality: derivation and validation. PLoS Med. 2013;10(12):e1001577; discussion e.
22. Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. Nat Rev Immunol. 2005;5(4):331-42.
23. Silk E, Zhao H, Weng H, Ma D. The role of extracellular histone in organ injury. Cell Death Dis. 2017;8(5):e2812.
24. Ryckman C, Vandal K, Rouleau P, Talbot M, Tessier PA. Proinflammatory activities of S100: proteins S100A8, S100A9, and S100A8/A9 induce neutrophil chemotaxis and adhesion. J Immunol. 2003;170(6):3233-42.
25. Yu M, Wang H, Ding A, Golenbock DT, Latz E, Czura CJ, et al. HMGB1 signals through toll-like receptor (TLR) 4 and TLR2. Shock. 2006;26(2):174-9.
26. Karikó K, Ni H, Capodici J, Lamphier M, Weissman D. mRNA is an endogenous ligand for Toll-like receptor 3. J Biol Chem. 2004;279(13):12542-50.
27. Giordano L, Gregory AD, Pérez Verdaguer M, Ware SA, Harvey H, DeVallance E, et al. Extracellular release of mitochondrial DNA: triggered by cigarette smoke and detected in COPD. Cells. 2022;11(3).
28. Krishnaswamy JK, Chu T, Eisenbarth SC. Beyond pattern recognition: NOD-like receptors in dendritic cells. Trends Immunol. 2013;34(5):224-33.
29. Roh JS, Sohn DH. Damage-associated molecular patterns in inflammatory diseases. Immune Netw. 2018;18(4):e27.
30. Wang B, Han J, Elisseeff JH, Demaria M. The senescence-associated secretory phenotype and its physiological and pathological implications. Nat Rev Mol Cell Biol. 2024;25(12):958-78.
31. Coppé JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu Rev Pathol. 2010;5:99-118.
32. Takasugi M, Yoshida Y, Ohtani N. Cellular senescence and the tumour microenvironment. Mol Oncol. 2022;16(18):3333-51.
33. Shimada K, Crother TR, Karlin J, Dagvadorj J, Chiba N, Chen S, et al. Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. Immunity. 2012;36(3):401-14.
34. Lin X, Zhou Y, Xue L. Mitochondrial complex I subunit *MT-ND1* mutations affect disease progression. Heliyon. 2024;10(7):e28808.
35. Zhang Q, Itagaki K, Hauser CJ. Mitochondrial DNA is released by shock and activates neutrophils via p38 map kinase. Shock. 2010;34(1):55-9.
36. Bliksøen M, Mariero LH, Torp MK, Baysa A, Ytrehus K, Haugen F, et al. Extracellular mtDNA activates NF-κB via toll-like receptor 9 and induces cell death in cardiomyocytes. Basic Res Cardiol. 2016;111(4):42.
37. Liao Z, Tong B, Ke W, Yang C, Wu X, Lei M. Extracellular vesicles as carriers for mitochondria: biological functions and clinical applications. Mitochondrion. 2024;78:101935.
38. Vikramdeo KS, Anand S, Khan MA, Khushman M, Heslin MJ, Singh S, et al. Detection of mitochondrial DNA mutations in circulating mitochondria-originated extracellular vesicles for potential diagnostic applications in pancreatic adenocarcinoma. Sci Rep. 2022;12(1):18455.

39. Byappanahalli AM, Noren Hooten N, Vannoy M, Mode NA, Ezike N, Zonderman AB, et al. Mitochondrial DNA and inflammatory proteins are higher in extracellular vesicles from frail individuals. Immun Ageing. 2023;20(1):6.
40. Di Florio DN, Sin J, Coronado MJ, Atwal PS, Fairweather D. Sex differences in inflammation, redox biology, mitochondria and autoimmunity. Redox Biol. 2020;31:101482.
41. Koupenova M, Clancy L, Corkrey HA, Freedman JE. Circulating platelets as mediators of immunity, inflammation, and thrombosis. Circ Res. 2018;122(2):337-51.
42. Lee JH, Shim YR, Seo W, Kim MH, Choi WM, Kim HH, et al. Mitochondrial double-stranded RNA in exosome promotes interleukin-17 production through toll-like receptor 3 in alcohol-associated liver injury. Hepatology. 2020;72(2):609-25.
43. Kim KM, Meng Q, Perez de Acha O, Mustapic M, Cheng A, Eren E, et al. Mitochondrial RNA in Alzheimer's disease circulating extracellular vesicles. Front Cell Dev Biol. 2020;8:581882.
44. Hough KP, Trevor JL, Strenkowski JG, Wang Y, Chacko BK, Tousif S, et al. Exosomal transfer of mitochondria from airway myeloid-derived regulatory cells to T cells. Redox Biol. 2018;18:54-64.
45. He C, Bassik MC, Moresi V, Sun K, Wei Y, Zou Z, et al. Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. Nature. 2012;481(7382):511-5.
46. Laker RC, Drake JC, Wilson RJ, Lira VA, Lewellen BM, Ryall KA, et al. AMPK phosphorylation of Ulk1 is required for targeting of mitochondria to lysosomes in exercise-induced mitophagy. Nat Commun. 2017;8(1):548.
47. Halling JF, Pilegaard H. Autophagy-dependent beneficial effects of exercise. Cold Spring Harb Perspect Med. 2017;7(8).
48. Kwon I, Lee Y, Cosio-Lima LM, Cho JY, Yeom DC. Effects of long-term resistance exercise training on autophagy in rat skeletal muscle of chloroquine-induced sporadic inclusion body myositis. J Exerc Nutrition Biochem. 2015;19(3):225-34.
49. Guan Y, Drake JC, Yan Z. Exercise-induced mitophagy in skeletal muscle and heart. Exerc Sport Sci Rev. 2019;47(3):151-6.
50. Eltboli O, Bafadhel M, Hollins F, Wright A, Hargadon B, Kulkarni N, et al. COPD exacerbation severity and frequency is associated with impaired macrophage efferocytosis of eosinophils. BMC Pulm Med. 2014;14:112.
51. Hodge S, Hodge G, Ahern J, Jersmann H, Holmes M, Reynolds PN. Smoking alters alveolar macrophage recognition and phagocytic ability: implications in chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol. 2007;37(6):748-55.
52. McCubbrey AL, Curtis JL. Efferocytosis and lung disease. Chest. 2013;143(6):1750-7.
53. Wootton DG, Diggle PJ, Court J, Eneje O, Keogan L, Macfarlane L, et al. Recovery from pneumonia requires efferocytosis which is impaired in smokers and those with low body mass index and enhanced by statins. Thorax. 2016;71(11):1052-4.
54. Vandivier RW, Henson PM, Douglas IS. Burying the dead: the impact of failed apoptotic cell removal (efferocytosis) on chronic inflammatory lung disease. Chest. 2006;129(6):1673-82.
55. Cohen PL, Caricchio R, Abraham V, Camenisch TD, Jennette JC, Roubey RA, et al. Delayed apoptotic cell clearance and lupus-like autoimmunity in mice lacking the C-mer membrane tyrosine kinase. J Exp Med. 2002;196(1):135-40.
56. Khanna S, Biswas S, Shang Y, Collard E, Azad A, Kauh C, et al. Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice. PLoS One. 2010;5(3):e9539.
57. Leung A, Gregory NS, Allen LH, Sluka KA. Regular physical activity prevents chronic pain by altering resident muscle macrophage phenotype and increasing interleukin-10 in mice. Pain. 2016;157(1):70-9.
58. Poon IK, Lucas CD, Rossi AG, Ravichandran KS. Apoptotic cell clearance: basic biology and therapeutic potential. Nat Rev Immunol. 2014;14(3):166-80.
59. Simpson RJ, Lowder TW, Spielmann G, Bigley AB, LaVoy EC, Kunz H. Exercise and the aging immune system. Ageing Res Rev. 2012;11(3):404-20.
60. Donovan T, Bain AL, Tu W, Pyne DB, Rao S. Influence of exercise on exhausted and senescent T Cells: a systematic review. Front Physiol. 2021;12:668327.
61. Shimizu K, Suzuki N, Imai T, Aizawa K, Nanba H, Hanaoka Y, et al. Monocyte and T-cell responses to exercise training in elderly subjects. J Strength Cond Res. 2011;25(9):2565-72.
62. Spielmann G, McFarlin BK, O'Connor DP, Smith PJ, Pircher H, Simpson RJ. Aerobic fitness is associated with lower proportions of senescent blood T-cells in man. Brain Behav Immun. 2011;25(8):1521-9.
63. Xu M, Tchkonia T, Ding H, Ogrodnik M, Lubbers ER, Pirtskhalava T, et al. JAK inhibition alleviates the cellular senescence-associated secretory phenotype and frailty in old age. Proc Natl Acad Sci U S A. 2015;112(46):E6301-10.
64. Robbins PD, Jurk D, Khosla S, Kirkland JL, LeBrasseur NK, Miller JD, et al. Senolytic drugs: reducing senescent cell viability to extend health span. Annu Rev Pharmacol Toxicol. 2021;61:779-803.
65. Whitham M, Parker BL, Friedrichsen M, Hingst JR, Hjorth M, Hughes WE, et al. Extracellular vesicles provide a means for tissue crosstalk during exercise. Cell Metab. 2018;27(1):237-51.e4.
66. Chong MC, Shah AD, Schittenhelm RB, Silva A, James PF, Wu SSX, et al. Acute exercise-induced release of innate immune proteins via small extracellular vesicles changes with aerobic fitness and age. Acta Physiol (Oxf). 2024:e14095.
67. Vechetti IJ, Jr., Valentino T, Mobley CB, McCarthy JJ. The role of extracellular vesicles in skeletal muscle and systematic adaptation to exercise. J Physiol. 2021;599(3):845-61.
68. Xhuti D, Nilsson MI, Manta K, Tarnopolsky MA, Nederveen JP. Circulating exosome-like vesicle and skeletal muscle microRNAs are altered with age and resistance training. J Physiol. 2023;601(22):5051-73.
69. Wang T, Zhang Y, Taaffe DR, Kim JS, Luo H, Yang L, et al. Protective effects of physical activity in colon cancer and underlying mechanisms: a review of epidemiological and biological evidence. Crit Rev Oncol Hematol. 2022;170:103578.
70. Apostolopoulou M, Mastrototaro L, Hartwig S, Pesta D, Straßburger K, de Filippo E, et al. Metabolic responsiveness to training depends on insulin sensitivity and protein content of exosomes in insulin-resistant males. Sci Adv. 2021;7(41):eabi9551.
71. Castaño C, Mirasierra M, Vallejo M, Novials A, Párrizas M. Delivery of muscle-derived exosomal miRNAs induced by HIIT improves insulin sensitivity through down-regulation of hepatic FoxO1 in mice. Proc Natl Acad Sci U S A. 2020;117(48):30335-43.
72. Gallo-Villegas J, Aristizabal JC, Estrada M, Valbuena LH, Narvaez-Sanchez R, Osorio J, et al. Efficacy of high-intensity, low-volume interval training compared to continuous aerobic training on insulin resistance, skeletal muscle structure and function in adults with metabolic syndrome: study protocol for a randomized controlled clinical trial (Intraining-MET). Trials. 2018;19(1):144.

73. Abbasi A, Hutchins E, Meechoovet B, Hall M, Garcia-Mansfield K, Soriano A, et al. Greater enrichment of biological pathways in circulating extracellular vesicles of controls than COPD following exercise. Eur Respir J. 2023;62(suppl 67):PA2273.
74. Bhattacharya P, Dhawan UK, Hussain MT, Singh P, Bhagat KK, Singhal A, et al. Efferocytes release extracellular vesicles to resolve inflammation and tissue injury via prosaposin-GPR37 signaling. Cell Rep. 2023;42(7):112808.
75. Panahi S, Yazdi Z, Sanchouli M, Sajadi F, Motavasselian F, Maghareh Abed H, et al. The role of extracellular vesicles in efferocytosis. Mini Rev Med Chem. 2024;24(6):634-41.
76. Ma Z, Sun Y, Yu Y, Xiao W, Xiao Z, Zhong T, et al. Extracellular vesicles containing MFGE8 from colorectal cancer facilitate macrophage efferocytosis. Cell Commun Signal. 2024;22(1):295.
77. Abbasi A, Vitorino SA, Okerblom J, Hertz JPZ, Wolf A, Indralingam C, et al. Proteomic analysis unveiled exercise-induced changes in extracellular vesicles from BALF And blood In cigarette smoke exposed mice: 3095. Medi Sc Sports Exerc. 2024;56(10S):1122-3.
78. Galvan MD, Foreman DB, Zeng E, Tan JC, Bohlson SS. Complement component C1q regulates macrophage expression of Mer tyrosine kinase to promote clearance of apoptotic cells. J Immunol. 2012;188(8):3716-23.
79. Benoit ME, Clarke EV, Morgado P, Fraser DA, Tenner AJ. Complement protein C1q directs macrophage polarization and limits inflammasome activity during the uptake of apoptotic cells. J Immunol. 2012;188(11):5682-93.
80. Hulsebus HJ, O'Conner SD, Smith EM, Jie C, Bohlson SS. Complement component C1q programs a pro-efferocytic phenotype while limiting TNFα production in primary mouse and human macrophages. Front Immunol. 2016;7:230.
81. Alsina D, Lytovchenko O, Schab A, Atanassov I, Schober FA, Jiang M, et al. FBXL4 deficiency increases mitochondrial removal by autophagy. EMBO Mol Med. 2020;12(7):e11659.
82. Subramaniam S. Exaggerated mitophagy: a weapon of striatal destruction in the brain? Biochem Soc Trans. 2020;48(2):709-17.
83. Kubli DA, Gustafsson Å B. Mitochondria and mitophagy: the yin and yang of cell death control. Circ Res. 2012;111(9):1208-21.
84. Mizumura K, Cloonan SM, Nakahira K, Bhashyam AR, Cervo M, Kitada T, et al. Mitophagy-dependent necroptosis contributes to the pathogenesis of COPD. J Clin Invest. 2014;124(9):3987-4003.
85. Hariharan N, Ikeda Y, Hong C, Alcendor RR, Usui S, Gao S, et al. Autophagy plays an essential role in mediating regression of hypertrophy during unloading of the heart. PLoS One. 2013;8(1):e51632.
86. Wong CK, Smith CA, Sakamoto K, Kaminski N, Koff JL, Goldstein DR. Aging impairs alveolar macrophage phagocytosis and increases influenza-induced mortality in mice. J Immunol. 2017;199(3):1060-8.

Exercise-Induced Metabolic Reprogramming and Immune Modulation: A Novel Strategy for Cancer Therapy

Anqi He[1#], Sha Wang[1#], Tianjie Bao[1], Shunzi Rong[2], Chong Li[1], Min Luo[3], Chengqi He[1], Yonghong Yang[1], Yong Xia[1*]

[1] Rehabilitation Medicine Center and Institute of Rehabilitation Medicine, Key Laboratory of Rehabilitation Medicine in Sichuan Province, West China Hospital, Sichuan University, Chengdu, 610041, China
[2] West China School of Pharmacy, Sichuan University, Chengdu 610041, China
[3] Department of Biotherapy, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, China

ABSTRACT

Exercise represents a non-pharmacological strategy capable of concurrently modulating tumor metabolism and immunity. Regular physical activity reprograms systemic and tumor-localized metabolic networks, including glucose, lactate, amino acid, and lipid pathways, while enhancing innate and adaptive immune responses. Exercise-induced myokines (e.g., IL-6, IL-15, irisin, SPARC) and improved vascularization contribute to reshaping the tumor microenvironment (TME), mitigating immunosuppressive metabolite accumulation, and promoting T cell and NK cell infiltration. Mechanistically, exercise activates integrated signaling networks including AMPK–mTOR–HIF1α, PGC-1α–ERRα, and IL-6/STAT3 axes, supporting metabolic flexibility and anti-tumor immunity. Translational and clinical studies suggest exercise can enhance chemotherapy and immunotherapy efficacy, while precision exercise prescriptions based on FITT principles, biomarkers, and patient-specific tolerance may maximize therapeutic benefits. This review summarizes the molecular and systemic mechanisms of exercise-induced metabolic-immune reprogramming and outlines strategies for clinical translation in oncology.

INTRODUCTION

Cancer is a multifactorial disease driven by intricate genetic, epigenetic, and environmental influences. Among its hallmarks, metabolic reprogramming and immune evasion are recognized as key contributors to tumor initiation, progression, and resistance to therapy (1, 2). Tumor cells undergo profound metabolic adaptations to sustain rapid proliferation and survive under nutrient-deprived, hypoxic conditions. These changes—encompassing glycolysis, amino acid metabolism, and lipid utilization—reshape the TME and directly impair the function of various immune cell types, including $CD8^+$ T cells, Natural killer (NK) cells, and dendritic cells (DCs), thereby facilitating immune escape (3-5).

Central to tumor metabolic reprogramming is the Warburg effect, wherein cancer cells favor aerobic glycolysis, leading to excessive lactate production and acidification of the TME (6, 7). Lactate accumulation not only suppresses effector T and NK cell function but also promotes regulatory T cell differentiation and tumor-associated macrophage polarization (8). Similarly, altered amino acid metabolism (e.g., glutamine, serine, tryptophan) and lipid metabolism contribute to immune dysfunction and support tumor growth (4, 9, 10).

The immunosuppressive TME created by these metabolic shifts represents a major barrier to effective anti-tumor immunity and limits the efficacy of immunotherapies (11-14). As a result, targeting the metabolism-immunity axis is gaining traction as a promising therapeutic strategy (15).

Exercise, a well-established modulator of systemic metabolism and immune function, has recently emerged as a potential non-pharmacological intervention to disrupt tumor metabolic networks and restore immune competence (16-20). Studies suggest that exercise may improve glucose and lipid metabolism, reduce lactate accumulation, and reprogram amino acid utilization within tumors. Concurrently, exercise enhances the mobilization and cytotoxicity of immune effector cells, modulates immune checkpoint expression, and may synergize with immunotherapy (16-20).

**Corresponding author:*
[#]These authors contributed equally to this work.
[*]Correspondence:
Yong Xia (yxia4@scu.edu.cn).

However, important knowledge gaps remain. The impact of exercise varies across tumor types and individual patients due to molecular and physiological heterogeneity (21, 22). Moreover, the optimal parameters of exercise—type, duration, intensity—required to achieve therapeutic benefits in cancer contexts are not yet fully defined (22, 23).

This review aims to provide a comprehensive overview of how tumor metabolic reprogramming drives immune suppression and how exercise may counteract these effects through systemic and tumor-local immunometabolic modulation. We further explore emerging evidence supporting the integration of exercise into anti-cancer strategies and highlight directions for future research focused on precision exercise oncology.

HALLMARKS OF TUMOR METABOLISM: MECHANISMS AND IMPACT OF METABOLIC REPROGRAMMING

Tumor cells are distinguished by their profound metabolic alterations, a phenomenon first noted by Warburg, where cancer cells preferentially produce energy via glycolysis rather than relying on mitochondrial oxidative phosphorylation (OXPHOS)—even under aerobic conditions (24). This metabolic shift, commonly known as the Warburg effect, leads to an increased rate of glucose uptake and lactate production, providing the essential building blocks and energy required for rapid proliferation (24-27). The excessive lactate accumulation in the TME not only fuels tumor growth but also contributes to acidification, which in turn supports immune evasion and promotes angiogenesis (7, 8).

Beyond glycolysis, tumors exhibit extensive reprogramming of other metabolic pathways. Alterations in amino acid metabolism—such as the enhanced utilization of glutamine, serine, and glycine—enable cancer cells to replenish substrates for biosynthesis and sustain energy production even under nutrient-limited conditions (28-30). Similarly, modifications in lipid metabolism, including increased uptake and oxidation of fatty acids (FAs) and cholesterol, further support the structural and energetic needs of malignant cells (28, 29). These adaptive changes collectively not only meet the metabolic demands of tumor cells but also reshape the surrounding microenvironment to favor tumor survival and progression.

Moreover, the metabolic reprogramming of cancer cells has broader implications. The interplay between altered cancer cell metabolism and the diverse cell types within the TME—ranging from immune cells to stromal cells—creates a competitive landscape for nutrients. This competition can exacerbate immune suppression by depriving effector cells of vital metabolic substrates, thereby reinforcing mechanisms of immune escape (31, 32). Understanding these metabolic adaptations is therefore critical, as they reveal potential targets for therapies aimed at disrupting the tumor's metabolic network and restoring anti-tumor immunity (13-15).

IMMUNOMETABOLIC INTERACTIONS: HOW TUMOR-DERIVED METABOLITES REGULATE TUMOR PROGRESSION AND IMMUNE FUNCTION

Mounting evidence indicates that tumor metabolic reprogramming not only facilitates malignant cell survival and proliferation under hostile microenvironmental conditions but also promotes immune evasion, thereby accelerating tumor progression (15, 33, 34). Within the TME, cancer cells and immune cells coexist in a metabolically competitive and often suppressive landscape. Tumor-derived metabolites, such as glucose, lactate, and amino acids, play dual roles: they sustain cancer cell metabolism while simultaneously impairing anti-tumor immunity.

In this section, we examine how these metabolites influence immune cell function, alter metabolic pathways within the TME, and contribute to immunosuppression. We further explore strategies that may restore immune function by targeting these metabolic vulnerabilities (Figure 1 and Table 1).

GLUCOSE AND LACTATE: DUAL ROLES IN FUELING TUMORS AND SUPPRESSING IMMUNITY

Glucose metabolism: Competition between tumor and immune cells in the TME

Glucose is a critical energy substrate for both tumor cells and immune cells. Cancer cells reprogram their glucose metabolism by enhancing glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway (PPP), and the serine synthesis pathway (SSP), thereby supporting biosynthesis and proliferation (68). This metabolic flexibility enables tumor cells to adapt to hypoxia and nutrient stress, in part by maintaining transcriptional programs such as the yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) axis that drive oncogenic growth and invasiveness (69).

However, immune cells within the TME—particularly $CD8^+$ T cells and tumor-associated macrophages (TAMs)—also depend on glucose to sustain their effector functions (34, 70). Studies have shown that TAMs, especially the M2-like subtype, consume substantial amounts of glucose, promoting pro-tumoral behavior via O-GlcNAcylation of cathepsin B and facilitating chemoresistance and metastasis (70). In addition, tumor-derived metabolites such as D-2-hydroxyglutarate (D-2HG), produced through mutant isocitrate dehydrogenase (IDH), can impair glycolysis in $CD8^+$ T cells, suppressing their proliferation and cytotoxicity (71).

Pharmacologic or genetic interventions that inhibit glycolysis in tumor cells can, paradoxically, restore immune surveillance. For instance, inhibition of Glucose Transporter 1 (Glut1) enhances Cytotoxic T Lymphocyte (CTL) -mediated Tumor Necrosis Factor-alpha (TNFα) release and tumor cell lysis (39). Moreover, GLUT10 has been identified as a selective regulator of glucose uptake and effector function in $CD8^+$ T cells, making it a potential therapeutic target (35).

Table 1. Immunosuppressive effects of tumor-associated metabolites and their molecular targets in preclinical studies.

Metabolites	Immune Cells	Immune-Metabolic Interaction Targets	Effects/Mechanism on Immune-Metabolic Interaction	References
Glucose	CD8+ T cells	GLUT10	GLUT10 regulates glucose uptake and is essential for $CD8^+$ T-cell proliferation, effector function, and anti-tumor immunity.	(35)
Glucose	CD8+ T cells	NSUN2	Deletion of the glucose/NSUN2/TREX2 axis activates cGAS/STING signaling, increases $CD8^+$ T-cell infiltration, and enhances anti–PD-L1 response.	(36)
Glucose	MDM	GLUT1	PERK-driven glucose metabolism enhances macrophage immunosuppressive activity via histone lactylation, inhibiting T-cell function and promoting tumor growth.	(37)
Glucose	NK cell	MICA/B	High glucose inhibits MICA/B expression through the AMPK–Bmi1–GATA2 axis, reducing NK cell-mediated cytotoxicity.	(38)
Glucose	CTL	Glut1 and Gpi1	Deficiency of Glut1 or Gpi1 enhances cytotoxic T-cell–mediated killing of tumor cells.	(39)
Glucose	DC	STING signal	Glycolysis activates STING signaling in dendritic cells, enhancing their anti-tumor activity in lung cancer.	(40)
Glucose	DC	NCoR1	NCoR1 regulates the balance between tolerance and inflammation by modulating glycolysis and fatty acid oxidation in dendritic cells.	(41)
Glucose	B Cells	PP2A	PP2A redirects glucose metabolism from glycolysis to the PPP to protect B cells from oxidative stress.	(42, 43)
LA	CD8+ T cells	GLUT10	High lactate binds GLUT10 and blocks glucose uptake, suppressing $CD8^+$ T-cell activation and effector function.	(35)
LA	MDM, T cells	PERK-ATF4 axis	Lactate induces histone lactylation and IL-10 expression in macrophages, suppressing T-cell anti-tumor activity.	(37)
LA	Treg	MCT1	Lactate enhances PD-1 expression on Tregs, reducing effector T-cell function and promoting immune escape.	(44)
LA	B cells, T cells	mTOR signaling pathway	Tumor-derived lactate enhances glycolysis and OXPHOS in B cells, leading to glucose deprivation and T-cell inhibition.	(45)
LA	M2 macrophages, CD8 T cells	Myc	Myc promotes lactate-induced Gpr132-dependent M2 polarization and suppresses $CD8^+$ T-cell function in ovarian cancer.	(46)
LA	NK cell	CaMKK2	Lactate regulates CaMKK2 expression, promoting NK cell survival and proliferation within tumors.	(47)
LA	NK cell	MCT1/4, GLUT1	Tumor-derived lactate lowers NK-cell intracellular pH, induces mitochondrial stress, and promotes apoptosis, impairing anti-tumor immunity.	(48)
Lactate	DC	SREBP2	Lactate activates the SREBP2 pathway in DCs, driving their transition to a regulatory phenotype and suppressing anti-tumor immunity.	(49)
Lactate	T cell	MCT4	Lactate accumulation through MCT4 inhibits T-cell function and anti-tumor responses.	(50)
Lactate	T+ ex cells	MCT11	Upregulation of MCT11 by lactate increases T-cell sensitivity to high-lactate TME, leading to T-cell exhaustion.	(51)
Sodium lactate	CD8+ T cell	Histone deacetylase	Sodium lactate enhances $CD8^+$ T-cell stemness and anti-tumor immunity in a pH-independent manner.	(52)
D-Lactate	macrophage	PI3K/AKT pathway	D-lactate interacts with TLR2/TLR9, inhibits PI3K–AKT, activates NF-κB, and promotes M1 polarization.	(53)
Asn	CD8+ T cell	NRF2	Asparagine restriction activates NRF2, enhances $CD8^+$ T-cell proliferation, and boosts anti-tumor immunity.	(54)
Leucine	T cell	LRIG1, VISTA	LRIG1 binds VISTA, suppressing TCR signaling and CTL amplification, leading to immune evasion.	(55)
Valine	CD8+ T cell	HLA-A2	Valine enhances $CD8^+$ T-cell recognition of tumor cells lacking TAP function via improved HLA-A2 binding.	(56)
Glutamine	CD8+ T cells	CircTRPS1/miR-141-3p/GLS1 axis	Tumor exosomal circTRPS1 modulates glutamine metabolism and ROS homeostasis, limiting $CD8^+$ T-cell exhaustion.	(57)
Glutamine	cDC1s, CD8 T cell	SLC38A2	Glutamine supports cDC1–$CD8^+$ T-cell metabolic crosstalk to sustain anti-tumor immunity.	(58)
Glutamine	T cell and NK cell	TNF-α, IL2	Glutamine supplementation enhances T-cell activation, NK-cell cytotoxicity, and apoptosis in tumor-bearing mice.	(59)
Kynurenine	T cell	D-kynurenine	D-kynurenine modulates T-cell metabolism and proliferation, limiting immunosuppression.	(60)
Tryptophan	CD8+ T cell	AhR	Intratumoral I3A from tryptophan metabolism activates AhR and enhances the efficacy of immune checkpoint therapy.	(61)
Serine	Regulatory T Cell	GSH	Serine regulates GSH synthesis and one-carbon metabolism, modulating Treg suppressive capacity.	(62)
Arginine	TAM, CD8+ T cell	Collagen	Arginine depletion by TAMs promotes fibrosis and suppresses $CD8^+$ T-cell responses, reducing immunotherapy efficacy.	(63)
Fatty acid	Regulatory T Cells	PI3K-AKT-mTOR signaling pathway	RHOA mutations increase fatty acid production, enhancing Treg accumulation and immune suppression.	(64)
Fatty acid	CTL	FAO, CPT1A	Fatty acids inhibit CPT1A, reducing T-cell cytotoxicity and promoting immune evasion.	(65)
Cholesterol	Mφ	ABCA1	Cholesterol accumulation promotes immunosuppressive macrophage differentiation in tumors.	(66)

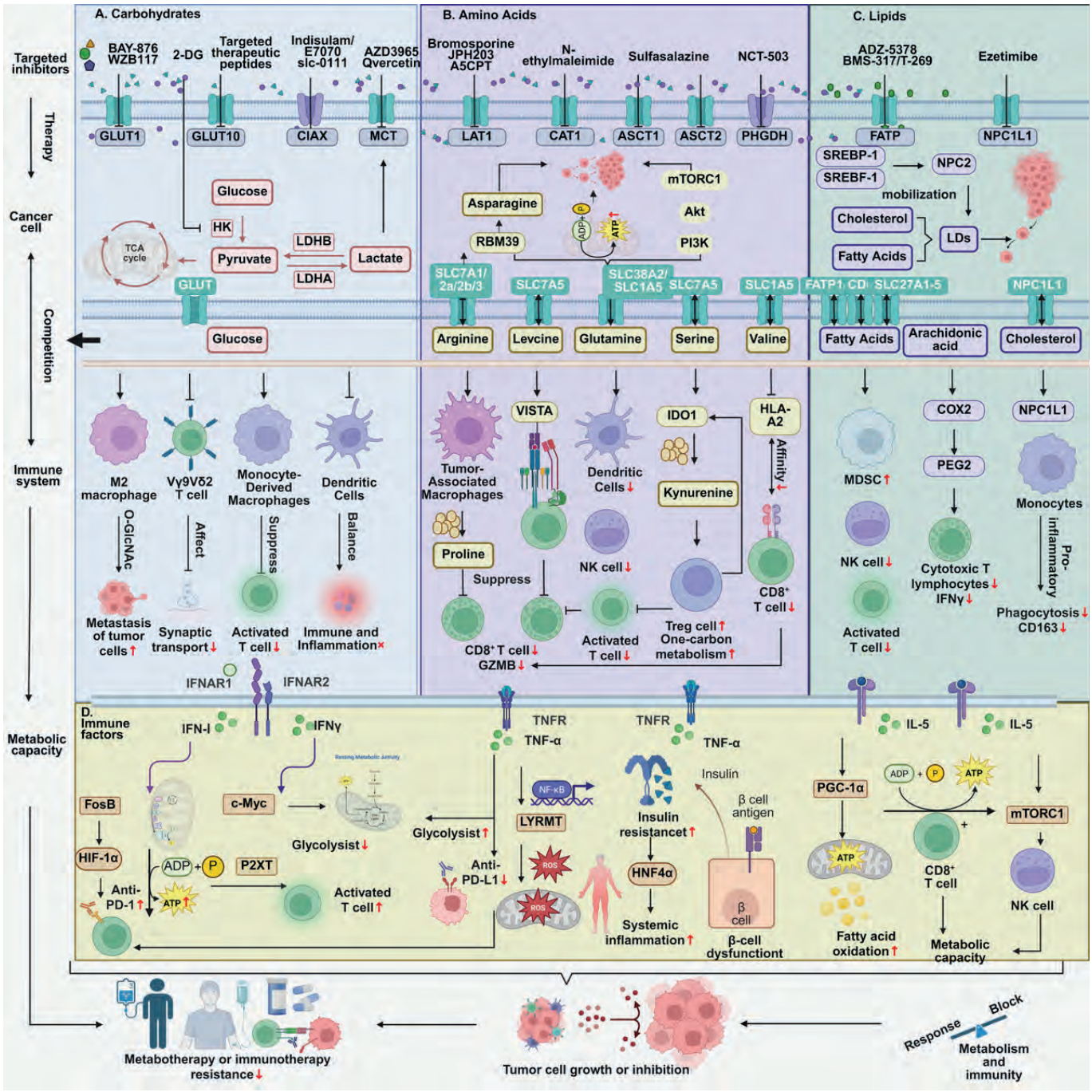


Figure 1. Metabolic reprogramming in tumors and its bidirectional crosstalk with the immune system.
(A) Carbohydrate metabolism: Tumor cells consume excessive glucose via GLUT transporters, converting it to lactate through aerobic glycolysis. Lactate suppresses T and NK cell function, promotes M2 macrophage polarization, and facilitates immune evasion. Inhibitors such as 2-DG and BAY-876 target glycolysis and glucose transport. (B) Amino acid metabolism: Tumor cells exploit glutamine, arginine, and tryptophan to support proliferation and evade immunity. Enzymes like IDO1 degrade tryptophan to kynurenine, activating Tregs and suppressing CD8+ T cells. Targeted therapies (e.g., Sulfasalazine, NCT-503) aim to restore immune function.
(C) Lipid metabolism: Tumor cells uptake fatty acids and cholesterol to fuel membrane synthesis and survival under stress. Lipid byproducts suppress NK and T cell activity through pathways such as COX2/PGE2. Inhibitors like Ezetimibe and other lipid regulators can improve immune responses.
(D) Immune Factors: Cytokines such as IFNγ, TNF, and IL-6 reshape cellular metabolism, which in turn modulates immune function. For instance, IFNγ enhances glycolysis in effector T cells, TNF affects lipid metabolism in macrophages, and IL-6 drives tumor glycolytic flux—all contributing to the balance between anti- and pro-tumor immune responses
Abbreviations: GLUT1: Glucose Transporter 1; GLUT10: Glucose Transporter 10; CAIX: Carbonic Anhydrase IX; MCT: Monocarboxylate Transporter; LAT1: L-type Amino Acid Transporter 1; CAT1: Cationic Amino Acid Transporter 1; ASCT1: Alanine: Serine: Cysteine Transporter 1; ASCT2: Alanine: Serine: Cysteine Transporter 2; PHGDH: 3-Phosphoglycerate Dehydrogenase; FATP: Fatty Acid Transport Protein; NPC1L1: Niemann-Pick C1-Like 1 Protein; SREBP-1: Sterol Regulatory Element-Binding Protein 1; SREBF-1: Sterol Regulatory Element-Binding Factor 1; LDs: Lipid Droplets; COX2: Cyclooxygenase 2; M2 macrophage: M2-Type Macrophage; Vγ9Vδ2 T cell: Vγ9Vδ2 T cell; DCs: Dendritic Cells; NK cell: Natural Killer Cell; Treg cell: Regulatory T Cell; CD8+ T cell: CD8 Positive T Cell; HLA-A2: Human Leukocyte Antigen A2; IDO1: Indoleamine 2,3-Dioxygenase 1; GZMB: Granzyme B; IFNγ: Interferon Gamma; PGE2: Prostaglandin E2; MDSCs: Myeloid-Derived Suppressor Cells; TCA cycle: Tricarboxylic Acid Cycle (Krebs Cycle); HK: Hexokinase; LDHA: Lactate Dehydrogenase A; LDHB: Lactate Dehydrogenase B; PI3K: Phosphoinositide 3-Kinase; mTORC1: Mammalian Target of Rapamycin Complex 1; Akt: Protein Kinase B; TNF: Tumor Necrosis Factor; IL-5: Interleukin-5

Future studies should explore how exercise impacts glucose availability and utilization in both tumor and immune cells, particularly regarding GLUT transporter expression, glycolytic flux, and nutrient competition in the TME (36-38, 40-43).

Lactate:
A key mediator of immune suppression and tumor survival
Lactate, the terminal product of aerobic glycolysis, has emerged as a multifunctional metabolite central to tumor growth and immune evasion. Tumor cells produce lactate primarily via lactate dehydrogenase A (LDHA), and extracellular lactate is imported through monocarboxylate transporters such as Monocarboxylic acid transporter 1 (MCT1) and MCT4 (8). Far from being a mere waste product, lactate supports cancer cell survival as a metabolic substrate and functions as a signaling molecule that shapes the TME.

One of the most critical roles of lactate is its capacity to regulate protein function through post-translational modification. Accumulated lactate can enter the nucleus and participate in lactylation, a novel epigenetic modification that influences protein stability, DNA repair, and transcriptional regulation (72, 73). In lung cancer cells, lactate-induced lactylation of autophagy-related proteins such as Unc-51 Like Autophagy Activating Kinase 1 (ULK1), Phosphatidylinositol 3-Kinase Catalytic Subunit Type 3 (PIK3C3), and UV Radiation Resistance Associated Gene (UVRAG) promotes endosomal-lysosomal degradation pathways, contributing to tumor adaptation and growth under stress conditions (74).

Lactate also plays a major role in shaping the immunosuppressive TME. It impairs the cytotoxic function of $CD8^+$ T cells by disrupting pyruvate metabolism, reduces NK cell viability, and promotes polarization of TAMs toward an M2 phenotype (35, 50, 52). Additionally, lactate can upregulate immunosuppressive cytokines like IL-10 and TGF-β, further reinforcing immune evasion. These effects collectively contribute to diminished anti-tumor immune surveillance and enhanced tumor progression.

Interestingly, the immunoregulatory effects of lactate appear to vary depending on pH. In tumor tissues, cancer cells highly express LDHA and produce large amounts of lactate through glycolysis. This process is accompanied by H^+ extrusion, leading to acidic lactate accumulation that acidifies the TME and suppresses anti-tumor immunity (75). Among these factors, acidity is a dominant feature of the TME—it can inhibit cytosolic extracellular signal–regulated kinase (ERK) activity, block oncogene-induced mitochondrial fragmentation, and promote mitochondrial fusion. Consequently, it enhances mitochondrial respiration and supports cancer cell adaptation to various metabolic stresses, allowing sustained proliferation even under harsh conditions (76). At physiological pH, lactate primarily exists in its anionic form ($lactate^-$), and sodium lactate does not contribute to acidification. Notably, lactate can serve as a metabolic substrate for $CD8^+$ T cells, promoting oxidative metabolism and thereby enhancing T cell stemness and persistence (77). For instance, sodium lactate—administered in a neutral-pH form—has been shown to promote $CD8^+$ T cell stemness and improve anti-tumor immunity in preclinical models (52). Intraperitoneal injection of sodium lactate (2 g/kg) in mice reduces tumor growth in breast cancer, cutaneous melanoma, Lewis lung cancer (LLC) and colon adenocarcinoma models, and this effect is dependent on T cells (78). Helene et al. also reported that in a mouse breast cancer model, 2 g/kg sodium lactate treatment increased the number of tumor-infiltrating immune cells, specifically $CD3^+$ T cells, including $CD4^+$ and $CD8^+$ T cells (79). In contrast, administration of a lower dose (1 g/kg) of sodium lactate shortened the survival of tumor-bearing mice (79). Therefore, it is essential to distinguish between the immunosuppressive effects driven by acidic lactate accumulation and the immunoenhancing effects induced by neutral sodium lactate supplementation (44-49, 51, 53).

In summary, lactate is a central mediator linking tumor metabolism with immune dysfunction (Figure 2). Future studies should investigate how exercise modulates lactate dynamics, particularly its influence on lactylation, immune cell metabolism, and lactate transporter expression (e.g., MCT1/MCT4). Understanding these mechanisms may provide new opportunities to exploit exercise as an adjuvant strategy in metabolic-immunotherapy.

Amino acid metabolism: Modulating tumor growth and immune cell activity
Amino acids are essential substrates for cell growth, functioning as both nitrogen and carbon donors in biosynthetic pathways, signal transduction, and epigenetic regulation. Tumor cells exploit amino acid metabolism to bypass nutrient limitations and sustain rapid proliferation. Key amino acids involved include glutamine, arginine, tryptophan, serine, glycine, and asparagine (Asn), all of which contribute to tumor biosynthesis and redox balance (80, 81). Glutamine, although classified as non-essential, becomes conditionally essential for many tumors, especially under hypoxic conditions where it replaces glucose as a primary carbon source in the TCA cycle (82). Altered glutamine metabolism activates oncogenic pathways such as PI3K/AKT/mTOR, promoting tumor progression, as shown in hepatocellular carcinoma models (83). Similarly, high arginine levels drive metabolic remodeling and support tumor proliferation (84).

Immune cells also depend on amino acids for survival, proliferation, and effector function. $CD8^+$ T cells, macrophages, and neutrophils require distinct amino acid pools to execute anti-tumor responses (55-63) . For example, restricting Asn triggers NRF2-mediated stress responses that reduce glucose and glutamine consumption while boosting nucleotide synthesis, thereby enhancing $CD8^+$ T cell proliferation and cytotoxicity (54). Moreover, tryptophan catabolism via kynurenine—a ligand for the aryl hydrocarbon receptor (AhR)—promotes differentiation of immunosuppressive regulatory T cells (Tregs), dampening inflammation and immune surveillance (61).

Future research should examine how exercise influences amino acid metabolism in both tumor and immune compartments. Understanding exercise-induced changes in glutamine, arginine, and tryptophan pathways may provide novel opportunities to overcome metabolic suppression and improve anti-tumor immunity.

Lipid metabolism:
Supporting tumor adaptation and immune escape
Lipids serve multiple biological functions including energy stor-

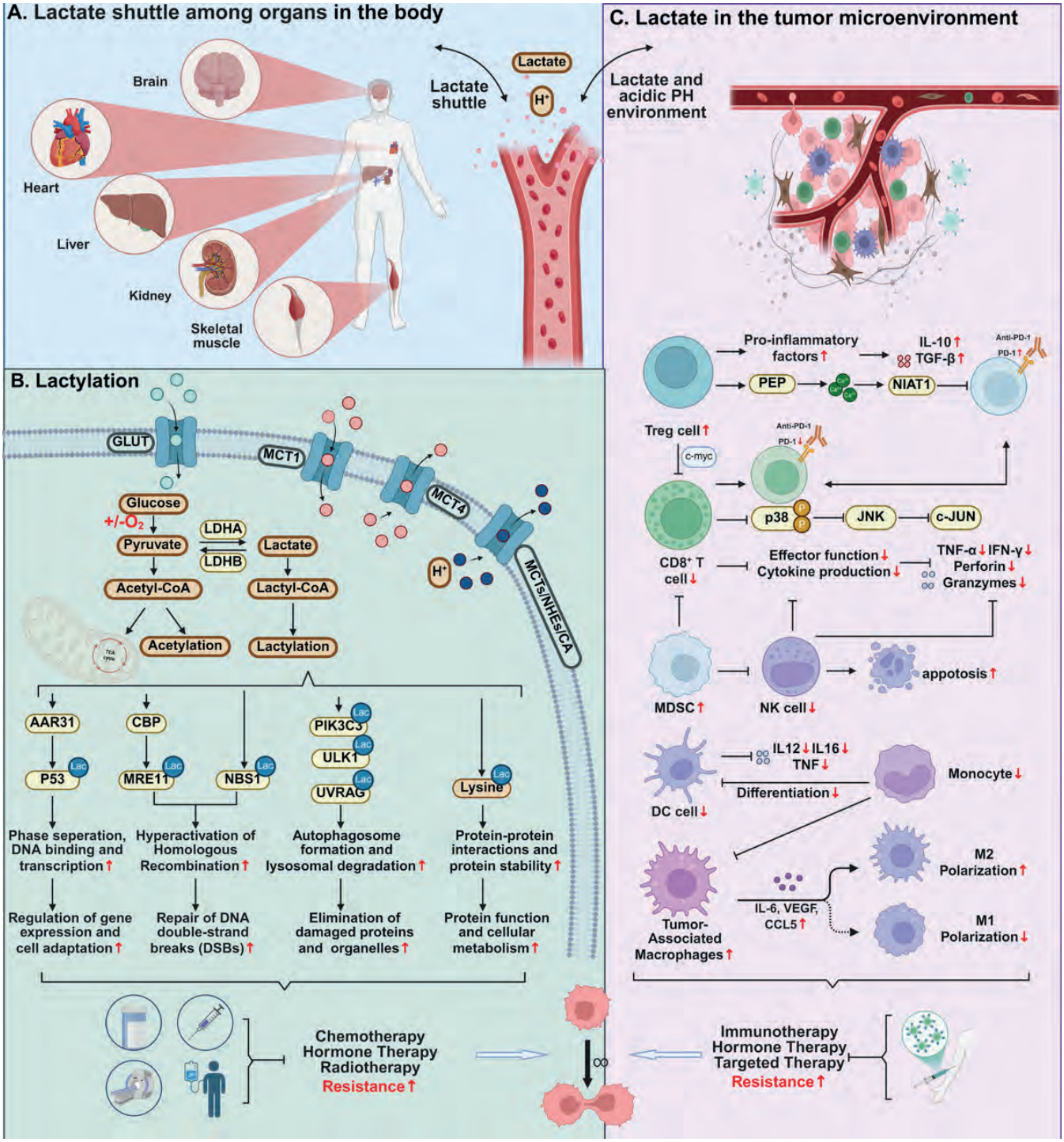


Figure 2. Multifaceted roles of lactate in tumor progression and immune suppression.
This figure summarizes the pleiotropic effects of lactate in the TME, highlighting its metabolic, signaling, and immunoregulatory functions.
(A) Lactate shuttle. Tumor cells generate lactate via LDHA and export it through MCT1/4 transporters. Lactate circulates between cells and organs, serving as a substrate and signaling molecule.
(B) Lactylation modification. Lactate-mediated protein lactylation represents a novel post-translational modification mechanism that extensively participates in critical biological processes including gene expression regulation, DNA damage repair, and protein stability maintenance. This modification not only provides survival and proliferation advantages for tumor cells but more importantly, induces resistance to chemotherapy, radiotherapy, and targeted therapies, thereby significantly enhancing tumor immune evasion. Research indicates that lactylation modification can alter the immunogenicity of tumor cells through epigenetic regulation, serving as a crucial molecular bridge connecting aberrant tumor metabolism with immunosuppressive microenvironments.
(C) Lactate in the TME. High lactate concentrations acidify the TME, upregulate immunosuppressive cytokines (e.g., IL-10, TGF-β), and impair $CD8^+$ T cell and NK cell function. Lactate also promotes polarization of tumor-associated macrophages (TAMs) toward an M2 phenotype and increases Treg recruitment.
Overall, lactate acts as a central mediator of metabolic-immune crosstalk, shaping an immune-suppressive and tumor-promoting environment.
Abbreviations: GLUT: Glucose Transporter; MCT1: Monocarboxylate Transporter 1; MCT2: Monocarboxylate Transporter 2; MCT4: Monocarboxylate Transporter 4; LDHA: Lactic Acid Dehydrogenase A; LDHB: Lactic Acid Dehydrogenase B; Acetyl-CoA: Acetyl Coenzyme A; Lactyl-CoA: Lactyl Coenzyme A; AARS1: Alanyl-tRNA Synthetase 1 ; CBP: CREB Binding Protein; NBS1: Nibrin Protein 1; PIK3C3: Phosphoinositide 3-Kinase Catalytic Subunit 3; UVRAG: UV Radiation Resistance-Associated Gene Protein; IL-10: Interleukin 10; TGF-β: Transforming Growth Factor Beta; TNF-α: Tumor Necrosis Factor Alpha; IFN-γ: Interferon Gamma; IL-12: Interleukin 12; IL-16: Interleukin 16; IL-6: Interleukin 6; VEGF: Vascular Endothelial Growth Factor; ROS: Reactive Oxygen Species; SOD1: Superoxide Dismutase 1; SOD2: Superoxide Dismutase 2.

age, membrane synthesis, and intracellular signaling. In cancer, dysregulated lipid metabolism—particularly involving FAs and cholesterol—is a hallmark of tumor progression (85). Tumor cells exploit lipid pathways to meet increased energy demands, enhance membrane biosynthesis, and support metastatic potential under fluctuating nutrient availability in the TME (86). Upregulation of lipid-regulatory transcription factors such as SREBF1 and SREBP-1 promotes autophagy-mediated lipid droplet breakdown, enabling efficient mobilization of cholesterol and FAs for membrane synthesis and rapid growth (87).

Lipid metabolism is also reprogrammed in stromal and immune cells within the TME. FAs enhance OXPHOS in resting DCs, skew macrophage polarization, suppress cytokine production in NK cells, and contribute to T cell exhaustion (65, 66). Cholesterol accumulation in the TME further promotes immune dysfunction by inducing T cell depletion (64, 67).

Despite increasing insights, the precise mechanisms through which lipid metabolism affects immune modulation remain poorly defined. Future studies should explore how exercise modifies lipid metabolic pathways in both tumors and immune cells. Investigating fatty acid oxidation (FAO), lipid signaling, and membrane remodeling in response to physical activity could reveal novel therapeutic strategies for restoring immune competence in cancer.

IMMUNOMETABOLIC INTERACTIONS: IMPACT OF IMMUNE-DERIVED FACTORS ON TUMOR METABOLISM.

Immune-derived factors, such as interferons (IFNs), TNF-α, and interleukin-15 (IL-15), act as key mediators of immunometabolic reprogramming within the TME. These signaling molecules not only modulate immune responses but also remodel tumor cell metabolism, collectively influencing tumor progression and therapeutic outcomes.

IFN:
Driven Crosstalk Between Tumor Metabolism and Immune Editing

IFNs are broadly classified into type I and type II families. Type I IFNs include IFN-α, IFN-β, and IFN-ω, which all signal through a common cell-surface receptor, whereas IFN-γ is the sole type II interferon that engages a distinct receptor complex (88). Type I interferons (IFN-Is) are central coordinators of tumor–immune interactions(88). One preclinical study has shown that persistent IFN-I signaling and the chronic activation of interferon-stimulated genes (ISGs) may paradoxically promote tumor progression. For instance, the ISG mediates the pro-tumorigenic effects of IFN-I by enhancing mRNA translation and reprogramming lipid metabolism, thereby driving cancer growth (89). Conversely, IFN-I can also induce tumoricidal metabolic responses. One study demonstrated that IFN-I promotes OXPHOS and triggers autophagy-dependent Adenosine triphosphate (ATP) release, with extracellular ATP subsequently activating P2X7-dependent dendritic cell signaling, leading to enhanced anti-tumor T cell responses (90). Beyond its classic immune-modulatory roles, IFN-α has been shown to suppress the key metabolic regulator FBJ murine osteosarcoma viral oncogene homolog B (FosB) in an Interferon regulatory factor 1 (IRF1)-dependent manner, concurrently inhibiting downstream HIF-1α signaling. This downregulation of glycolysis-related genes sensitizes hepatocellular carcinoma cells to anti–PD-1 immunotherapy, providing a promising combinatorial strategy for advanced liver cancer (91). IFN-γ, a hallmark cytokine produced by activated T cells, profoundly shapes tumor metabolic phenotypes. In melanoma models, T cell–derived IFN-γ upregulates c-Myc expression in tumor cells, driving metabolic reprogramming that favors immune evasion (92). Intriguingly, recent evidence suggests that IFN-γ signaling may paradoxically activate tumor-intrinsic compensatory pathways during immune checkpoint blockade. IFN-γ–induced secretion of fibroblast growth factor 2 (FGF2) promotes PKM2 phosphorylation, inhibits glycolysis, and lowers intracellular NAD^+ levels. The resulting IFNγ–FGF2–β-catenin axis integrates immune, metabolic, and proliferative pathways, representing a potential biomarker and therapeutic target to prevent hyperprogressive disease (HPD) during immunotherapy (93).

TNF-α:
Linking inflammation, mitochondrial bioenergetics, and immune suppression

TNF-α acts as a central regulator of both metabolism and inflammation within the TME, where it exhibits paradoxical roles. Preclinical research indicates that TNF-α–mediated activation of NF-κB regulates the expression of LYRM7, affecting mitochondrial supercomplex assembly and reactive oxygen species (ROS) production—key drivers of metabolic reprogramming (94). Moreover, TNF-α derived from TAMs can enhance tumor glycolysis and upregulate PD-L1 expression, thereby diminishing the efficacy of PD-L1 blockade therapy (95). Beyond cancer, macrophage-secreted TNF-α and IL-1β contribute to insulin resistance and β-cell dysfunction, establishing a mechanistic link between systemic metabolic disorders and chronic inflammation (96). In early-stage breast cancer, infiltration of innate immune cells secreting IL-6 and TNF-α into the liver suppresses HNF4α, leading to hepatic metabolic reprogramming and systemic inflammation that together promote tumor initiation (97).

IL-15:
Sustaining lymphocyte metabolic fitness and therapeutic persistence

IL-15, a γc-family cytokine produced by bone marrow, dendritic, and muscle cells, is a master regulator of T cell and NK cell homeostasis, effector function, and memory formation. In addition to these immunologic roles, IL-15 enhances mitochondrial biogenesis through activation of the PGC-1α pathway, increases spare respiratory capacity (SRC), and promotes FAO via upregulation of CPT1A, collectively favoring oxidative metabolism over glycolysis (98). IL-15–induced memory-like $CD8^+$ T cells display enhanced OXPHOS and mitochondrial fusion mediated by cardiolipin remodeling (98, 99). In adoptive cell therapy, IL-15–expanded CAR-T cells (CAR-T/IL15) retain a stem cell–like memory phenotype (Tscm) characterized by $CD62L^+CD45RA^+CCR7^{+-}$ expression, reduced exhaustion, and improved mitochondrial fitness. Mechanistically, IL-15–mediated suppression of mTORC1 activity maintains metabolic quiescence while enhancing in vivo antitumor efficacy.

IL-15 also modulates NK cell metabolism and anti-cancer

activity. High IL-15 levels stimulate NK cell metabolism through mTOR activation, boosting cytotoxic function (100). However, dosing strategies are critical: continuous IL-15 exposure induces NK cell exhaustion, functional decline, and reduced fatty acid metabolism (101). Together, these findings highlight IL-15 as a pivotal regulator of immune cell metabolic resilience, offering a promising avenue for optimizing next-generation immunotherapies (102).

EXERCISE-INDUCED REGULATION OF TUMOR METABOLISM AND IMMUNE RESPONSES: MECHANISMS AND THERAPEUTIC POTENTIAL

Emerging evidence highlights the capacity of exercise to modulate both systemic and tumor-localized metabolism, while simultaneously reprogramming immune responses. These dual effects position exercise as a promising adjunctive approach to cancer therapy (103, 104). Regular physical activity has been shown to inhibit tumor growth by altering tumor cell metabolic networks, enhancing immune surveillance, and improving treatment responsiveness (105).

Mechanistically, exercise impacts tumor progression through three major pathways: (1) regulation of tumor metabolite availability (e.g., glucose, lactate, FAs), (2) enhancement of innate and adaptive immune cell function, and (3) modulation of immune-metabolic interactions within the TME. Exercise reprograms systemic energy metabolism and modulates cytokines involved in tumor biology, such as interleukin-6 (IL-6) (106-108). The exercise-induced modulation of metabolic hormones and their impact on tumor signaling pathways are discussed in the following section.

Moreover, endurance training has been shown to alter expression of lactate metabolism-related factors (e.g., MCT1, LDHA, Estrogen-Related Receptor Alpha (ERRα)), reduce tumor acidity, and restore immune cell infiltration (109). Myokines such as IL-6, irisin, and Secreted Protein Acidic And Cysteine Rich (SPARC), secreted by skeletal muscle during exercise, further modulate tumor metabolism, epithelial–mesenchymal transition (EMT), and immune activation (110, 111).

As such, understanding the mechanisms of exercise-induced metabolic reprogramming may lead to novel intervention strategies that reshape the TME, limit nutrient competition, and restore anti-tumor immunity. This section explores how these systemic and local effects translate into potential clinical benefits.

Systemic effects of exercise on organ metabolism and immune homeostasis

The anti-tumor effects of exercise are partly attributable to its ability to induce metabolic and immune remodeling across multiple organs. Key systems—including skeletal muscle, cardiovascular system, liver, and gastrointestinal tract—respond to exercise through metabolic reprogramming and immune regulation, which in turn can influence tumor progression (20, 112, 113). We have compiled a summary of this research in Figure 3.

As the primary site for nutrient storage and energy utilization, skeletal muscle plays a central role in the physiological adaptations to exercise, particularly in the regulation of metabolism, angiogenesis, and immune responses (114). A single bout of exercise can directly enhance amino acid and glucose uptake in skeletal muscle, while during the recovery phase, it promotes muscle protein synthesis and improves insulin-stimulated glucose disposal (115).

Exercise-induced improvements in cardiorespiratory fitness (CRF) are mediated by multiple mechanisms, including modulation of sympathetic nerve activity, increased myocardial contractility, reduced oxidative stress, promotion of physiological cardiac hypertrophy, enhancement of stroke volume and heart rate, and regulation of non-coding RNAs and their signaling pathways (116, 117). A preclinical study demonstrated that exercise elevates the abundance and/or activity of antioxidant enzymes—such as superoxide dismutases (SOD1, SOD2), glutathione peroxidase, and catalase—in mitochondria isolated from ventricular cardiomyocytes. These changes reduce myocardial ROS and enhance cardiac antioxidant capacity (118)

The liver, as the major metabolic organ, also exhibits significant adaptive responses to exercise. Accumulating evidence indicates that regular physical activity improves systemic metabolism and insulin sensitivity, induces adaptations in glucose and lipid metabolism, and markedly reduces the risk of chronic metabolic diseases such as alcoholic fatty liver and metabolic dysfunction–associated fatty liver disease (MAFLD) (119-121). Exercise-induced fibronectin (FN1) acts as a circulating metabolic factor that triggers hepatic autophagy and systemic insulin sensitization via the hepatic α5β1 integrin–IKKα/β–JNK1–BECN1 signaling pathway, thereby promoting protein and organelle turnover and systemic metabolic adaptation (122).

The gut is a dynamic environment, and the gut microbiota plays an important role in human biology, including metabolism, endocrine, neuronal, and immune functions (123, 124). A recent study found that proper exercise can increase the diversity of gut microbes and the number and activity of beneficial bacteria, indirectly affecting the body's metabolism and immunity, thereby having a positive impact on health (123, 125).

Exercise regulation of tumor-associated metabolites and enzymes: pathways to enhanced anti-tumor immunity

Exercise represents a powerful, non-pharmacologic intervention capable of reshaping the tumor metabolic landscape and restoring immune competence. Tumor cells display altered metabolic profiles, with enhanced glycolysis, glutaminolysis, and lipid metabolism that facilitate rapid proliferation and immune evasion (6, 8, 28, 86). These same pathways, however, can be modulated by exercise to limit nutrient availability to tumor cells while supporting immune cell activation. The dual impact of exercise on metabolism and immunity makes it a unique tool in anti-cancer strategies, particularly in the context of immunotherapy (16, 103, 104) .

This section outlines how exercise regulates tumor metabolism (5.2.1), reshapes immune function (5.2.2), and exercise and immune-metabolic crosstalk: mechanistic integration of tumor suppression (5.2.3), highlighting the mechanistic foundations and therapeutic relevance of these interactions. We have compiled a

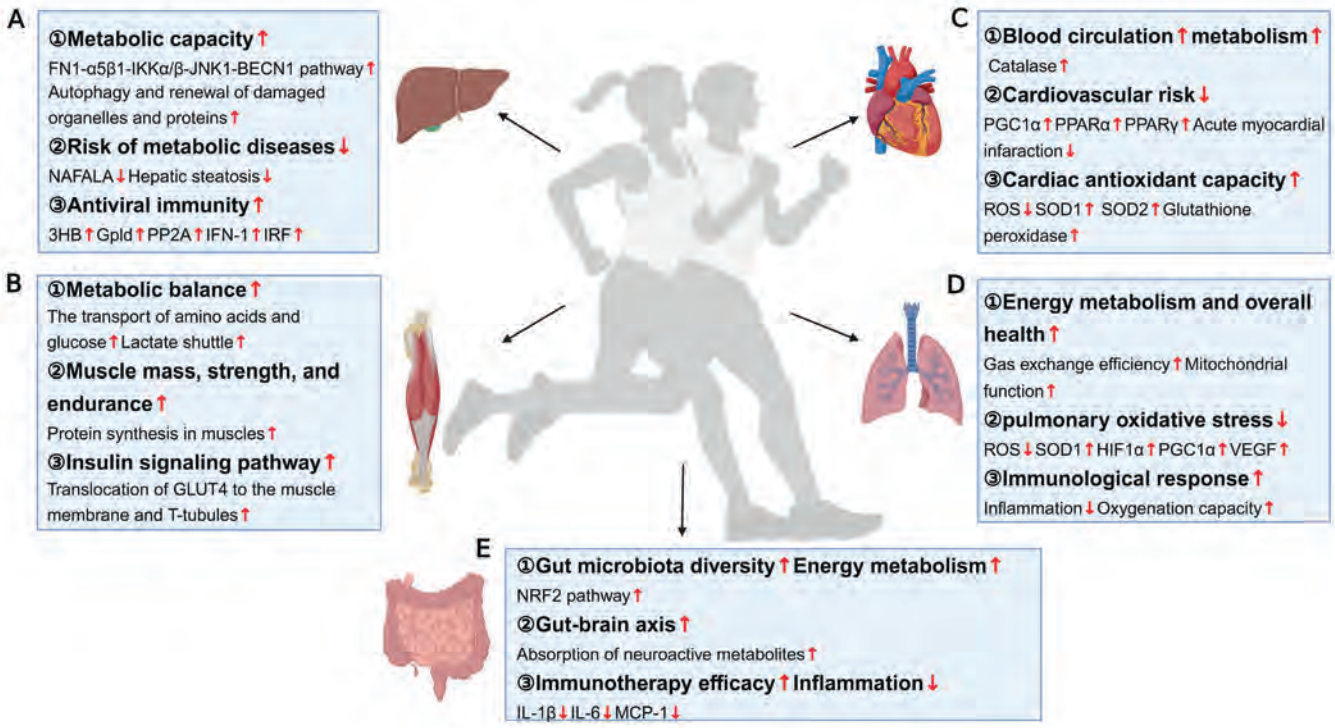


Figure 3. The effects of exercise on different organs.
(A) Exercise might improve liver metabolic function, promotes metabolic balance, and enhances gluconeogenesis and lipid metabolism.
(B) Exercise could enhance muscle metabolic function, improves energy metabolism and insulin sensitivity, and helps maintain muscle mass.
(C) Exercise probably improves blood circulation and metabolic capacity of the heart, promoting cardiovascular health.
(D) Exercise can improve lung function, increasing oxygen intake and carbon dioxide expulsion efficiency.
(E) Exercise may promote gut microbiome diversity, improves gut health and immune function, and reduces the risk of metabolic diseases.
Abbreviations: FN1-α5β1- IKKα/β-JNK1-BECN1 pathway: Fibronectin 1-α5β1-KKα/β-JNK1-Beclin1 autophagy-related protein pathway; Hepatic steatosis: Liver fat degeneration; 3HB: 3-Hydroxybutyrate; Gpld: Glycosylphosphatidylinositol-specific Phospholipase D; PP2A: Protein Phosphatase 2A; IFN-1: Interferon 1; IRF: Interferon Regulatory Factor; PGC1α: Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1 Alpha; PPARα: Peroxisome Proliferator-Activated Receptor Alpha; PPARγ: Peroxisome Proliferator-Activated Receptor Gamma; Acute myocardial infarction: Acute myocardial infarction (heart attack); ROS: Reactive Oxygen Species; SOD1: Superoxide Dismutase 1; SOD2: Superoxide Dismutase 2; Glutathione peroxidase: Glutathione Peroxidase; HIF1α: Hypoxia-Inducible Factor 1 Alpha; VEGF: Vascular Endothelial Growth Factor; IL-1β: Interleukin 1 Beta; IL-6: Interleukin 6; MCP-1: Monocyte Chemoattractant Protein 1; NRF2 pathway: Nuclear Factor E2-Related Factor 2 pathway

summary of this research in Table 2 and Figure 4.

Reprogramming tumor metabolism through exercise: Glucose, lactate, and growth factor pathways

Tumor cells rely on a high rate of aerobic glycolysis, consuming excessive glucose and secreting lactate into the TME. This metabolic phenotype—known as the Warburg effect—not only supports anabolic processes but also creates a hostile, acidic microenvironment that suppresses immune activity (6, 7). Lactate impairs T and NK cell functions, enhances regulatory T cell expansion, and polarizes macrophages toward immunosuppressive phenotypes (8, 35, 50).

Exercise can reverse several aspects of this metabolic imbalance. Endurance and moderate-intensity exercise improve whole-body glucose homeostasis, lower insulin and IGF-1 levels, and reduce the availability of metabolic substrates to tumors (106-108). GLUT4-mediated glucose uptake in skeletal muscle is enhanced during exercise, effectively reducing systemic glucose concentrations and limiting tumor access to this critical nutrient (134). Additionally, exercise modulates lactate dynamics within the TME (109). Furthermore, exercise may offer a non-pharmacologic strategy to mitigate lactate-mediated immune suppression. Preclinical studies have shown that endurance training reduces lactate accumulation in tumors by enhancing systemic lactate clearance and downregulating key lactate transport and metabolic regulators such as LDHA, MCT1, and ERRα (109). These adaptations can shift the TME from immunosuppressive to immune-permissive.

Beyond glycolysis, exercise also affects amino acid and lipid metabolism. For example, exercise reduces tumor glutamine uptake by downregulating glutamine transporters and modulating MYC- and mTOR-dependent pathways that drive glutaminolysis (82-84). These changes impair nucleotide synthesis and antioxidant defense in cancer cells. Similarly, exercise enhances FAO in multiple tissues and reduces lipid accumulation within tumors, decreasing the metabolic flexibility and survival advantage of cancer cells under stress (86, 87).

As a systemic modulator, exercise influences metabolic hormones, including insulin and IGF-1. Downregulation of the insulin/IGF-1 axis leads to inhibition of the PI3K/AKT/mTOR pathway, reducing cancer cell proliferation and resistance to apoptosis (107). These systemic effects synergize with local metabolic changes in the TME, collectively creating a metabolically hostile environment for tumors while preserving immune cell function.

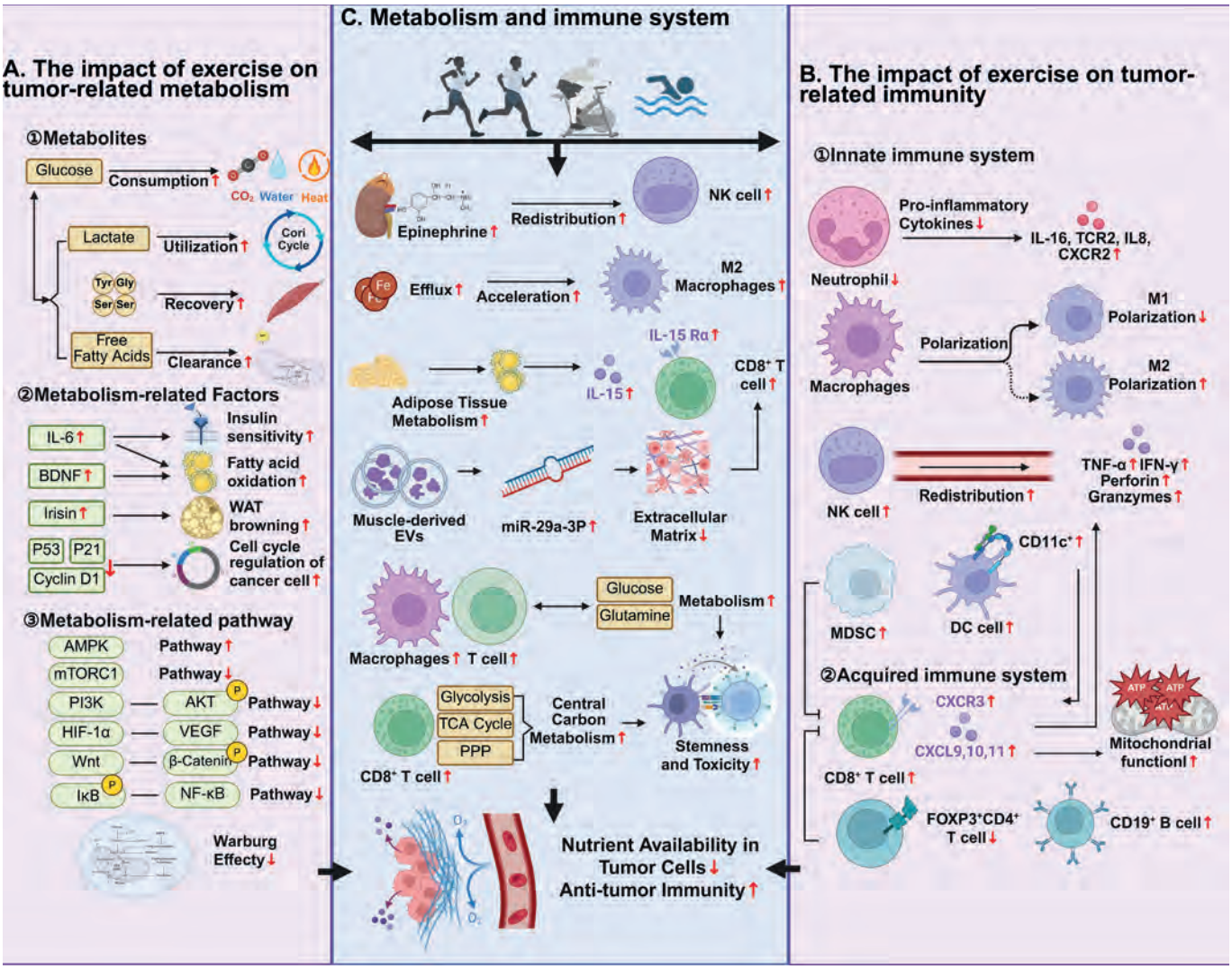


Figure 4. The potential mechanisms through which exercise affects tumors by regulating metabolism and immunity.
(A) Exercise Modulates Metabolic Pathways Exercise regulates the levels of metabolites such as glucose, lactate, and fatty acids, thereby increasing the metabolic rate of tumor-associated organs. This limits nutrient availability to tumors, reducing their risk and progression. Exercise also influences metabolism-related growth factors or cytokines, modulating tumor-related metabolic pathways to inhibit tumor cell proliferation and survival. (B) Exercise Modulates Immune Responses Exercise enhances tumor-associated immune responses by modulating the number and function of immune cells in both the innate and adaptive immune systems. This improvement strengthens anti-tumor immunity. In the innate immune system, exercise increases the activity of NK cells, neutrophils, macrophages, and dendritic cells. In the adaptive immune system, exercise enhances the function of T cells and B cells, promoting anti-tumor immune responses. (C) Exercise Modulates Immune-Metabolic Interactions Exercise reduces tumor immune escape and suppresses tumor progression by modulating immune-metabolic interactions. For instance, in animal models of pancreatic cancer, exercise promotes immune cell mobilization and tumor infiltration, enhancing the immune system's anti-tumor effects. Furthermore, exercise may affect tumor progression by modulating iron metabolism and tumor extracellular matrix. This includes selectively targeting tumor cells and promoting muscle-derived extracellular vesicles, such as miR-29a-3p, to inhibit the tumor extracellular matrix and facilitate immune cell infiltration.
Abbreviations: NK cell: Natural Killer Cell; M2 Macrophages: M2 Type Macrophages; CD8+ T cell: CD8 Positive T Cell; IL-15 Raβ: Interleukin 15 Receptor Alpha Chain; IL-15: Interleukin 15; miR-29a-3p: MicroRNA-29a-3p; EVs: Extracellular Vesicles; WAT: White Adipose Tissue; BDNF: Brain-Derived Neurotrophic Factor; P21: Cyclin-Dependent Kinase Inhibitor 1A; AMPK: AMP-Activated Protein Kinase; mTORC1: Mammalian Target of Rapamycin Complex 1; PI3K: Phosphoinositide 3-Kinase; HIF-1β: Hypoxia-Inducible Factor 1 Alpha; VEGF: Vascular Endothelial Growth Factor; Wnt: Wnt Signaling Pathway;β-Catenin: Beta-Catenin; IkB: Inhibitor of Nuclear Factor Kappa B; NF-βB: Nuclear Factor Kappa B; MDSCs: Myeloid-Derived Suppressor Cells; CXCR3: CXC Chemokine Receptor 3; CXCR9,10,11: CXC Chemokine Receptor 9, 10, 11; FOXP3+CD4+ T cell: Forkhead Box Protein P3 Positive CD4 Positive T Cell; CD19+ B cell: CD19 Positive B Cell; TNF-β: Tumor Necrosis Factor Alpha; IFN-β: Interferon Gamma

Table 2. Summary of the potential regulatory mechanisms of exercise on metabolic and immune function in preclinical models.

Cancer type	Research subject	Parameters of exercise	Metabolites	Immune cells	The effect of exercise on metabolic-immune regulation	References
NSCLC	Mouse	Voluntary wheel running	Collagen, miR-29a-3p	T cells	Voluntary exercise could increase T cell infiltration via the collagen inhibition orchestrated inflammatory TIME.	(126)
Melanoma Liver cancer, Lewis, Lung Cancer	Mouse	Voluntary wheel running	Epinephrine	NK cells	Regular exercise probably enhances immune function through epinephrine - and IL-6-mediated NK cell mobilization and infiltration, thereby inhibiting tumor growth and reducing tumor incidence and risk of recurrence.	(127)
Pancreatic cancer	Rat	Moderate Intensity Training Program (8 weeks)	Glucose and glutamine	Lymphocytes and macrophages	Moderate-intensity exercise can improve the survival of tumor-bearing rats by modulating glucose and glutamine metabolism in immune cells (e.g., lymphocytes and macrophages), promoting increased aerobic metabolism, and improving immune function.	(128)
Breast cancer	Mouse	Running wheels	TCA Metabolites	CD8 T cell	Exercise could alter the central carbon metabolism of CD8+ T cells by inducing the production and secretion of metabolites in skeletal muscle, which in turn enhances their antitumor effects.	(129)
Pancreatic cancer	Rat	High-intensity treadmill training (10 weeks, 5 days/week, 30 minutes/day, 85% VO2 max)	Glucose and glutamine	Lymphocytes and macrophages	High-intensity exercise would promote anti-tumor effects by modulating immune function and tumor cell metabolism (e.g., glucose and glutamine metabolism), and thus may be an effective strategy against cancer.	(130)
Lymphoma	Mouse	Voluntary wheel running	Epinephrine	NK cell	Voluntary wheel running could mobilize cytotoxic immune cells and prevents tumor progression through β2-adrenergic receptor signaling, suggesting that combining physical activity with adrenergic modulation can enhance anti-tumor immune responses.	(131)
GvL	Mouse	Voluntary wheel running	Genes related to metabolic processes	Lymphocytes	Voluntary wheel running could downregulates genes related to mitochondrial function, protein synthesis, and metabolism in tumors, and improves donor lymphocyte infusion outcomes by enhancing graft-versus-leukemia effects while reducing graft-versus-host disease, partially through metabolic reprogramming of leukemia cells	(132)
PDA	Mouse	Treadmill, 30min, per day at 15cm/s, 5days/week.	Epinephrine	CD8+ T cells	Exercise possibly promotes immune mobilization and accumulation of tumor-infiltrating IL15Ra+ CD8 T cells and inhibits tumor growth.	(133)

Collectively, these adaptations suggest that exercise reprograms the tumor's metabolic network, reducing nutrient access and interfering with biosynthetic pathways essential for malignant progression.

Modulation of innate and adaptive immunity by exercise

Exercise exerts robust immunomodulatory effects, influencing both the quantity and functionality of innate and adaptive immune cells. Acute exercise mobilizes immune cells into circulation, including NK cells, monocytes, neutrophils, and DCs, while chronic training induces sustained enhancements in immune surveillance and anti-tumor immunity (126-128, 131, 132) .

In the adaptive compartment, exercise improves the cytotoxicity of $CD8^+$ T cells by increasing granzyme B, perforin, and IFN-γ production, while reducing the expression of exhaustion markers like Programmed Death-1 (PD-1), TIM-3, and LAG-3 (19, 108). These functional improvements translate to enhanced killing of tumor cells and improved responsiveness to immune checkpoint blockade therapies. Exercise has also been shown to promote $CD8^+$ memory T cell differentiation and expansion, thereby establishing long-term immune protection (108, 110, 111).

NK cells are particularly sensitive to exercise stimuli. Physical activity increases NK cell mobilization and infiltration into tumors, improves their activation status, and enhances the release of cytolytic molecules such as FasL and TRAIL (126, 129). These changes contribute to both early detection and control of tumor growth.

Exercise also impacts myeloid cell populations in the TME. It can reduce the accumulation of immunosuppressive myeloid-derived suppressor cells (MDSCs), which otherwise inhibit T cell responses through arginase production and ROS generation (130). Moreover, exercise skews macrophage polarization from the M2 phenotype toward an M1-like, pro-inflammatory phenotype, enhancing antigen presentation and promoting anti-tumor immunity (126).

Importantly, exercise also improves the trafficking and infiltration of immune cells into tumors, a process often hindered by the dense extracellular matrix and abnormal vasculature. Exercise-induced vascular remodeling and reduced tumor fibrosis facilitate immune cell access and improve immune–tumor engagement (110, 111, 129).

These immunological benefits collectively enhance immune readiness and support long-term tumor control, particularly when combined with immunotherapies.

EXERCISE AND IMMUNE-METABOLIC CROSSTALK: MECHANISTIC INTEGRATION OF TUMOR SUPPRESSION

Macro-functional overview: Exercise reshapes tumor metabolism and immunity

The functional outcomes of anti-tumor immunity are intimately linked to metabolic support. Within the TME, nutrient competition between tumor and immune cells often leaves effector lymphocytes functionally impaired. For example, glucose depletion impairs T cell proliferation and cytokine secretion, while tryptophan degradation via IDO1 leads to Treg induction and $CD8^+$ T cell suppression (61).

Exercise can shift this metabolic competition in favor of the immune system. By reducing tumor consumption of glucose, glutamine, and lipids, exercise helps preserve these nutrients for immune cell activation (133). Moreover, exercise activates AMPK signaling in immune cells, enhancing their metabolic flexibility and mitochondrial function, allowing them to sustain cytotoxicity even under nutrient stress (135).

Exercise also attenuates tumor-derived suppressive metabolites such as lactate and kynurenine. Reduced lactate levels in the TME improve $CD8^+$ T cell viability, increase effector cytokine secretion, and inhibit lactate-mediated polarization of TAMs (8, 52). Meanwhile, suppression of tryptophan catabolism and kynurenine production by exercise may limit AhR signaling, reducing Treg differentiation and restoring immune surveillance (61).

Myokines secreted during physical activity—including IL-6, IL-7, IL-15, irisin, and SPARC—further modulate immune metabolism and function (110, 111). For example, IL-15, a key regulator of lymphocyte metabolic fitness as detailed in section 4.3, may mediate several exercise-induced benefits on immune cells. Irisin has been shown to inhibit tumor cell proliferation and EMT, and may play a role in modifying stromal cell behavior (110, 111).

Exercise-induced improvement in tumor vasculature, perfusion, and oxygenation also plays a vital role in immune–metabolic synergy. Better oxygen delivery reduces hypoxia-driven immunosuppression and restores OXPHOS in T cells. Combined with improved immune cell trafficking, these effects enhance the depth and durability of anti-tumor responses.

Together, these findings illustrate how exercise simultaneously remodels the metabolic and immunological landscape of tumors. A deeper understanding of this bidirectional regulation may facilitate the development of individualized exercise regimens as adjuncts to immunotherapy or metabolic-targeted cancer treatments.

AMPK–mTOR–HIF1α axis

AMPK serves as a central energy sensor and regulator of cellular homeostasis. The AMPK–mTOR–HIF1α signaling axis not only governs tumor metabolic plasticity but also enhances mitochondrial function in T cells and NK cells, thereby supporting immune effector activity and energy supply (136). One preclinical study demonstrates that regular exercise markedly activates AMPK and its downstream phosphorylation targets, leading to the suppression of tumor proliferation (137). Mechanistically, exercise-induced AMPK activation suppresses mTOR signaling, reduces tumor glycolysis and HIF1α-driven lactate accumulation, and enhances mitochondrial oxidative capacity in T and NK cells—forming a core framework of "energy sensing–metabolic reprogramming–immune activation"(138). In melanoma models, exercise improves tumor vascular architecture and oxygenation via an ERK5-dependent mechanism, which increases $CD8^+$ T-cell infiltration, reprograms myeloid cell phenotypes, and destabilizes HIF1α under reduced hypoxic stress (139). Similarly,

aerobic exercise improves tumor perfusion and oxygen supply while decreasing mitochondrial ROS accumulation, further promoting HIF1α inactivation (140). In breast cancer models, inhibition of the mTOR pathway by exercise reverses metabolic reprogramming, limits glucose and glutamine utilization, and induces tumor cell apoptosis (141).

At the systemic level, exercise also counteracts the proliferative stimulus of a high-fat diet. For instance, in MCF7 cells, exercise-activated AMPK upregulates p27, downregulates pAkt, and increases AdipoR1 expression, thereby promoting cell-cycle arrest in an intensity-dependent manner—high-intensity training (>3 km/day) completely abolishes HFD-induced proliferation (142). Furthermore, exercise enhances NK cell activity accompanied by upregulation of PIK3R1, which supports NK cell maturation, trafficking, and cytotoxic functions (143).

PGC-1α–ERRα axis

The PGC-1α–ERRα axis represents a crucial molecular bridge connecting exercise, mitochondrial function, and immunometabolic adaptation (144). PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha) is a master transcriptional coactivator regulating mitochondrial biogenesis and oxidative metabolism, and it plays a central role in muscle fiber type switching (145). Acting as a metabolic sensor of environmental stress (146), exercise-induced activation of PGC-1α enhances mitochondrial health, promotes OXPHOS, and maintains energy homeostasis (147). Beyond its direct metabolic role, PGC-1α regulates the secretion of myokines such as irisin and IL-15, which convey systemic signals that reshape the metabolic flexibility of tumor and immune cells (148). Through this endocrine-like mechanism, exercise not only improves systemic energy balance but also indirectly enhances the metabolic fitness of immune effectors. Within tumor cells, PGC-1α enhances OXPHOS and restrains glycolysis (149), while in skeletal muscle it contributes to whole-body metabolic stability (150).

Meanwhile, ERRα functions downstream of PGC-1α to control oxidative gene networks. Genetic enhancement of ERRα increases exercise endurance and fatigue resistance by globally remodeling DNA binding and transcriptional programs, leading to increased oxidative fiber proportion, mitochondrial biogenesis, FAO, and lactate homeostasis (151). Targeting ERRα has thus emerged as a strategy to develop exercise mimetics, which can improve mitochondrial respiration and mitigate age-related or disease-induced muscle dysfunction(152). Importantly, ERRα also regulates lactate and energy metabolism in tumors; exercise can inhibit its aberrant activation, reduce TME acidification, and restore immune cell function (153).

Together, these findings define the PGC-1α–ERRα axis as a mechanistic bridge between exercise, mitochondrial function, and immune metabolism, serving as a central node of exercise-mediated metabolic regulation in cancer.

IL-6/STAT3 axis

The IL-6/STAT3 pathway constitutes another key interface between exercise and immunometabolic regulation. In non-small cell lung cancer (NSCLC), sustained activation of IL-6–STAT3 signaling contributes to $CD8^+$ T-cell exhaustion and tumor progression (154). However, exercise modulates this pathway in an opposite, transient, and beneficial manner.

One clinical study has shown that resistance training increases plasma levels of anti-inflammatory cytokines and simultaneously activates STAT3 signaling in peripheral blood mononuclear cells (PBMCs), highlighting a potential mechanism by which resistance exercise enhances immune anti-inflammatory capacity (155). Exercise-induced IL-6 primarily acts as a myokine—a cytokine secreted from skeletal muscle—whose short-term elevation improves insulin sensitivity, activates STAT3-dependent mobilization pathways, and promotes the trafficking of NK and $CD8^+$ T cells from circulation to tissues (156). This transient cytokine surge represents a rapid immunometabolic mobilization mechanism underlying the systemic benefits of exercise.

During exercise, both skeletal muscle and stromal or immune cells within the TME release IL-6. Short-term elevations of myokine-derived IL-6 improve insulin sensitivity, stimulate anti-inflammatory cytokine production, recruit cytotoxic immune cells, and transiently activate IL-6 signaling within the TME. Long-term regular exercise lowers basal intratumoral IL-6, correlating with smaller tumor size, while maintaining metabolic and immune homeostasis (157-159).

The biological outcome of IL-6 signaling thus depends critically on exercise intensity and duration: moderate, transient IL-6 elevations are immunometabolically beneficial, whereas chronic or pathologically sustained IL-6 activation may promote tumor progression (160).

EXERCISE PRESCRIPTION PARAMETERS INFLUENCING TUMOR METABOLISM AND IMMUNITY

According to the FITT principle (Frequency, Intensity, Time, and Type), the design of exercise interventions should comprehensively consider key parameters such as exercise type, intensity, frequency, and duration. However, some patients with advanced malignancies may not tolerate evidence-based FITT protocols; therefore, exercise prescriptions should be adjusted according to individual physical capacity and treatment tolerance (161).

Different exercise types can activate distinct metabolic and immune signaling pathways to exert anticancer effects. Forced treadmill running—a common modality in preclinical studies—can regulate gut microbiota-derived metabolites such as formate, thereby enhancing tumor antigen-specific $CD8^+$ T-cell effector functions and improving the efficacy of immune checkpoint inhibitors (ICIs) through an Nrf2-dependent mechanism in melanoma (162).In contrast, voluntary wheel running elevates the level of skeletal muscle–derived extracellular vesicle (EV) miR-29a-3p, which targets COL1A1 to suppress collagen deposition in the tumor stroma, promoting T-cell infiltration and augmenting immunotherapy response (163).Resistance training improves metabolic health in cancer survivors by reducing adiposity and preserving lean muscle mass (164).In addition, traditional Eastern exercise modalities such as Taoist Qigong and Yoga also show immune-modulatory potential: Qigong alters B-cell and monocyte proportions, while Yoga reduces pro-inflammatory cytokines (TNF-α, IL-6), increases IL-10, and ameliorates oxidative stress (165).

Among the FITT components, intensity appears to be the most critical determinant of anticancer efficacy. Based on the percentage of maximal running capacity in mice, exercise can be classified as low (~30%), moderate (~60%), or high (~90%) intensity. Both low- and moderate-intensity training promote tumor vascular normalization; however, only moderate-intensity exercise significantly enhances intratumoral $CD8^+$ T-cell infiltration and effector molecule expression (CD69, IFNγ, GzmB) (166).A systematic review further demonstrated that a single bout of moderate-to-vigorous aerobic exercise could suppress tumor cell proliferation via increased secretion of myokines and hormones (167).Nonetheless, the benefits of intensity follow a U-shaped curve: excessive or exhaustive training may blunt immune surveillance. In a comparative preclinical study, high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) both improved cardiovascular fitness and quality of life in breast cancer patients, but no significant differences were observed in metabolic or inflammatory biomarkers (168).However, prolonged exhaustive high-intensity exercise (HIE) was shown to elevate anti-inflammatory cytokines and Treg proportions, potentially increasing infection susceptibility and impairing antitumor immunity (169).Collectively, these findings suggest that moderate-intensity exercise maximizes immune activation while minimizing immunosuppression, thereby representing the optimal balance for anticancer effects.

Exercise frequency also shapes metabolic and immune outcomes by regulating skeletal muscle circadian genes such as PPARδ. Regular training reinforces the synchronization between muscle clocks and metabolic pathways, improving glucose–lipid balance, mitochondrial efficiency, and redox homeostasis, while reducing tumor-related metabolic dysregulation (170).Epidemiological evidence indicates that total weekly exercise duration exceeding 280 minutes more effectively improves systemic inflammatory markers such as TNF-α and IL-8 (171).Moderate-to-high frequency programs (2–4 sessions per week) with moderate total load appear most efficient in eliciting short- to mid-term immune cell mobilization and metabolic benefits (171).After a single bout of training, leukocyte and NK-cell mobilization are positively correlated with intensity, and frequent repetition of such transient responses can lead to cumulative enhancement—an "EX-BOOST" phenomenon. Therefore, at least two sessions of moderate-to-vigorous exercise per week are recommended to maintain sustained immune activation (172).

Exercise duration determines whether adaptations remain transient or consolidate into long-term immunometabolic remodeling. A comprehensive review showed that short-term (4–12 weeks), medium-term (13–24 weeks), and long-term (>24 weeks) interventions each exert distinct physiological impacts (167).Short-term exercise primarily triggers acute leukocyte and NK-cell mobilization pathways (172) enhances glucose–lipid metabolism, and modulates myokine secretion (e.g., irisin, myostatin) (173). In contrast, long-term, regular training stabilizes immune homeostasis, reduces chronic inflammation, and enhances chemotherapy tolerance and efficacy (171).Immediately after acute exercise, serum IL-4, IL-6, and IL-10 levels rise transiently and return to baseline within one hour. Chronic moderate-to-high intensity training, however, decreases TNF-α, IL-6, and CD4/CD8 ratio, while increasing IL-10 after 24 weeks (174).These patterns suggest that long-term exercise establishes a persistent "immunometabolic memory" at the organ and systemic levels, and interventions lasting over 24 weeks may produce durable antitumor immune adaptations (175).

Overall, optimization of exercise prescription following the FITT framework is essential for achieving maximal therapeutic benefit in oncology. Exercise type defines the molecular entry point (e.g., Nrf2, myokine, or collagen remodeling pathways), intensity dictates the immune activation threshold, frequency governs rhythmic and cumulative adaptations, and duration determines the persistence of immunometabolic reprogramming. Properly calibrated, these parameters synergize to transform exercise into a form of precision metabolic–immune modulation, offering a non-pharmacological strategy to complement chemotherapy and immunotherapy in cancer treatment.

TRANSLATIONAL AND CLINICAL PERSPECTIVES: TOWARD PRECISION EXERCISE ONCOLOGY

With the rapid advancement of data analytics and personalized medicine, Precision Exercise Oncology is emerging as an important component of cancer rehabilitation and adjuvant therapy. The core principle is to design precise and dynamic exercise prescriptions based on tumor type, metabolic phenotype, immune status, and individual physiological characteristics, leveraging big data and intelligent monitoring to maximize patients' metabolic homeostasis and immune function (176).

Extensive clinical studies consistently indicate that exercise is safe and beneficial for most cancer patients, including those undergoing chemotherapy or radiotherapy (16-20).In parallel, preclinical evidence increasingly suggests that exercise combined with metabolic or immunotherapy can significantly enhance antitumor efficacy (133, 135).Currently, several clinical trials are investigating the synergistic effects of exercise with immuno- or metabolic therapies (Table 3), and a systematic review evaluating the role of exercise in modulating immunity and immunotherapy outcomes in cancer is forthcoming (177).However, most trials are ongoing, and the definitive therapeutic outcomes remain to be established.

Despite its potential benefits, exercise interventions must consider patients' physical capacity and metabolic reserve. Patients with cachexia, anemia, or immunosuppression may not tolerate standard exercise regimens. Given the variability in cancer types, treatment modalities, and potential cardiovascular risks, medical evaluation and clearance are generally required prior to initiating exercise programs.

For cachectic patients, a systematic review of randomized and non-randomized controlled trials indicated that exercise interventions are overall safe and can improve body composition and muscle strength (178). However, excessive whole-body exercise may exacerbate energy imbalance and weight loss in this population (179).Therefore, low-intensity resistance training combined with high-protein, energy-dense nutritional support, and dynamic adjustment of training loads based on body weight and muscle mass is recommended to ensure safety (180, 181).

For anemic patients, multiple studies demonstrate that aerobic exercise can maintain red blood cell levels and oxygen-carrying

Table 3. Summary of Ongoing Clinical Trials on Exercise Interventions Targeting Immune-Metabolic Regulation in Cancer.

Cancer Type	NCT Number	Immune-Metabolic Interaction	Exercise Intervention	Planned Enrollment	Expected Outcome / Endpoint
Melanoma	NCT06627595	Immunotherapy	Self-reported physical activity	160	Adverse events at treatment initiation and 6 months, assessed via IPAQ questionnaire
Urological Cancers	NCT06152926	Immunotherapy (e.g., ICI)	Regular physical activity or planned exercise	12	Exploratory study on patient perspectives and experiences regarding exercise during immunotherapy
Metastatic NSCLC	NCT04676009	Immunotherapy + Chemotherapy	Acute 35-min physical exercise	26	Feasibility of acute exercise immediately before immunotherapy and chemotherapy
Triple-Negative Breast Cancer	NCT06672120	Chemotherapy + Immunotherapy (checkpoint inhibitors)	24-week home-based, video-supervised endurance & resistance training	120	Incidence of CTRCD and ICI-related myocarditis at 24 and 52 weeks
Melanoma	NCT05358938	Checkpoint blockade immunotherapy	30-min moderate exercise	22	Exercise to enhance response to checkpoint blockade therapy
Lung Cancer	NCT06993896	Chemo- and/or Immunotherapy	Steps per week (walking)	38	Evaluate predictors and correlation with patient-reported outcomes (pain, distress, fatigue)
Cutaneous Cancers	NCT06008977	Checkpoint blockade immunotherapy	Moderate pace, 30 min/day	20	Feasibility of exercise intervention; preliminary data on day-of-therapy exercise effects
Hematological Malignancies	NCT05763563	CAR-T Cell Immunotherapy	Resistance training 30 min ×2/week + moderate aerobic ≥3 days/week	20	Evaluate exercise program for patients preparing for CAR-T therapy
Lung Cancer	NCT06026111	Immunotherapy	12-week training: HIIT, MICT, or usual care	30	Feasibility and comparison of immune activity, cardiorespiratory fitness, physical function, immunotherapy-related adverse events, and patient-reported outcomes
NSCLC	NCT06983899	Immunotherapy	Aerobic interval training	100	Aerobic interval training to improve oxygen utilization and immune activity, enhancing treatment response
Prostate Cancer	NCT03440879	ADT	Strength training	23	Assess ADT effects on basal muscle protein turnover and strength training response; secondary: postprandial glucose/insulin differences
Breast Cancer	NCT06928701	Nutritional supplement	Physical activity per WHO and EUSOMA criteria	160	Impact of targeted nutrition + exercise on metabolic and immune-related gene expression in early breast cancer patients undergoing neoadjuvant therapy
Prostate Cancer	NCT04870515	ADT	Aerobic + strength/ resistance exercise	40	Diet and exercise intervention to mitigate metabolic and physiologic changes caused by ADT and radiotherapy

capacity during breast cancer chemoradiotherapy. Moderate-intensity, regular aerobic exercise improves treatment-related anemia and encourages participation (182-186).Nevertheless, anemia severity, cancer type, treatment regimen, and exercise type and intensity all influence safety and tolerance (182).A systematic review suggested that platelet counts $>20 \times 10^9$/L are generally safe for exercise and daily activities, whereas $<10 \times 10^9$/L warrants caution or contraindication; hemoglobin <70–80 g/L may increase the risk of adverse events during exercise (187).Thus, pre-exercise evaluation of cardiopulmonary function and symptoms is essential for anemic patients, and HIE should be delayed if Hb <7–8 g/dL or hypoxic symptoms are present, with preference for static, breathing, or functional training modalities to ensure safety (161, 181).

Exercise has been shown to regulate the TME and enhance immune cell function, potentially ameliorating cancer-associated immunological dysfunction (188-190). Nevertheless, infection risk must be considered, particularly in immunocompromised patients such as those with HIV or post-transplant status (191). In these populations, exercise programs should be professionally supervised, prioritize low-intensity, progressive training, and adopt home-based or one-on-one formats, avoiding high-exposure environments while dynamically adjusting exercise load to optimize immune and metabolic benefits safely (192, 193).

Achieving truly precision exercise interventions requires the development of a biomarker-based framework that dynamically reflects metabolic and immune status, guiding exercise frequency, intensity, type, and duration, and predicting individual responsiveness. Although no standardized system exists, several candidate markers have been proposed:

Metabolic biomarkers: Lactate levels and LDHA expression may serve as immediate indicators of exercise intensity and tumor metabolic response, helping to avoid excessive acidification of the TME (194, 195). Circulating insulin concentrations provide long-term assessment of exercise tolerance and metabolic benefits (196). Exercise-induced changes in tryptophan metabolites (kynurenine) and gut microbiota-derived formate reflect modulation of $CD8^+$ T-cell function and immunosuppressive metabolic environment (162, 197). Mitochondrial regulator PGC-1α is implicated in the protective effects of physical activity on colorectal cancer, as its activity is positively stimulated by exercise and inversely correlated with cancer risk (144).

Immune biomarkers: Neutrophil-to-lymphocyte ratio (NLR), $CD4^+$, $CD8^+$, NK cells, Treg, and MDSCs—both absolute and relative levels—can identify patients suitable for exercise intervention and serve as dynamic predictors of response (198). Short-term exercise-induced immune activation can be monitored via IFN-γ, Granzyme B, and Perforin (199). Exercise mobilizes IL-15-dependent immune cells and promotes accumulation of tumor-infiltrating IL-15Rα^9 $CD8^+$ T cells, which exhibit potent antitumor activity, highlighting IL-15 as a potential therapeutic target in exercise oncology (133). Concurrently, exercise-induced IL-6 enhances insulin sensitivity, stimulates anti-inflammatory factor secretion, mobilizes immune cells, and reduces DNA damage, synergistically preventing tumor initiation and progression (200). Moreover, circulating NK cell activity, intratumoral NK cell infiltration, and IL-6 levels can serve as biomarkers of exercise-induced antitumor immune activation and may be used to monitor and optimize exercise interventions in cancer models (127).

In summary, precision exercise oncology offers a viable strategy for rehabilitation and adjuvant therapy in cancer patients, while providing new avenues for individualized optimization of metabolic and immune therapies. Future research should integrate multi-omics, physiological monitoring, and AI algorithms to construct a biomarker-feedback–based closed-loop exercise intervention system, achieving truly personalized and dynamically adaptive exercise prescriptions for cancer therapy.

CONCLUSION AND FUTURE PERSPECTIVES: EXERCISE AS A METABOLIC-IMMUNE MODULATOR IN CANCER THERAPY

Tumor metabolic reprogramming and immune evasion are two central hallmarks that synergistically sustain cancer progression and therapeutic resistance. As highlighted in this review, exercise represents a promising, low-cost, and non-pharmacological strategy capable of concurrently modulating tumor metabolism and enhancing anti-tumor immunity. By reshaping the tumor metabolic microenvironment—including reducing lactate accumulation, reprogramming amino acid and lipid metabolism, and improving systemic glucose homeostasis—exercise may indirectly restore immune function and strengthen host anti-tumor defenses.

Importantly, the influence of exercise extends beyond skeletal muscle to organs such as the liver, heart, and gut, each contributing to the metabolic and immune landscape of the TME. These systemic effects offer a unique opportunity to leverage exercise as an adjunct therapy in oncology.

However, significant challenges remain. Current knowledge on how exercise influences immune-metabolic pathways in different tumor types and molecular subtypes is still limited. Moreover, the optimal parameters of exercise—such as type, intensity, frequency, and duration—may vary significantly across patients, cancer types, and stages of treatment. Future studies should aim to establish precision exercise prescriptions that consider tumor heterogeneity, host fitness levels, and treatment context (e.g., immunotherapy vs. chemotherapy). Additionally, integrating wearable technology and real-time metabolic monitoring may enable dynamic adjustment of exercise protocols for individual patients.

In conclusion, exercise has the potential to become an integral component of personalized cancer therapy by targeting the immunometabolic vulnerabilities of tumors. Advancing this field requires translational and clinical research that bridges molecular mechanisms with practical implementation, ultimately enabling evidence-based, individualized exercise strategies in oncology care.

AUTHOR CONTRIBUTIONS

Anqi He and Sha Wang prepared tables and figures. Anqi He and Yong Xia conceptualized and wrote the manuscript. Shunzi Rong, Chong Li, Tianjie Bao, Min Luo, Chengqi He, and Yonghong Yang helped with conceptualization of the manuscript. All authors participated in manuscript editing and read and approved the final version.

FUNDING

This work was supported by Sichuan Science and Technology Program (Grant No. 2025ZNSFSC0559), Sichuan University Young Faculty Science and Technology Innovation Capability Enhancement Project (2024SCUOJTX046) and the National Natural Science Foundation of China (Grant No. 82573560).

Availability of data and materials
Not applicable.

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

REFERENCES

1. Stine ZE, Schug ZT, Salvino JM, and Dang CV. Targeting cancer metabolism in the era of precision oncology. Nature reviews Drug discovery 21: 141-162, 2022.
2. Galassi C, Chan TA, Vitale I, and Galluzzi L. The hallmarks of cancer immune evasion. Cancer cell 42: 1825-1863, 2024.
3. Faubert B, Solmonson A, and DeBerardinis RJ. Metabolic reprogramming and cancer progression. 368: eaaw5473, 2020.
4. Liu Y, Zhao Y, Song H, Li Y, Liu Z, Ye Z, Zhao J, Wu Y, Tang J, and Yao M. Metabolic reprogramming in tumor immune microenvironment: Impact on immune cell function and therapeutic implications. Cancer letters 597: 217076, 2024.
5. Bai R, Meng Y, and Cui J. Therapeutic strategies targeting metabolic characteristics of cancer cells. Critical reviews in oncology/hematology 187: 104037, 2023.
6. Huang K, Han Y, Chen Y, Shen H, Zeng S, and Cai C. Tumor metabolic regulators: key drivers of metabolic reprogramming and the promising targets in cancer therapy. Molecular cancer 24: 7, 2025.
7. Kooshan Z, Cárdenas-Piedra L, Clements J, and Batra J. Glycolysis, the sweet appetite of the tumor microenvironment. Cancer letters 600: 217156, 2024.
8. Wang ZH, Peng WB, Zhang P, Yang XP, and Zhou Q. Lactate in the tumour microenvironment: From immune modulation to therapy. EBioMedicine 73: 103627, 2021.
9. Wang Y, and Patti GJ. The Warburg effect: a signature of mitochondrial overload. Trends in cell biology 33: 1014-1020, 2023.
10. Chen J, Cui L, Lu S, and Xu S. Amino acid metabolism in tumor biology and therapy. Cell death & disease 15: 42, 2024.
11. Chew V, Toh HC, and Abastado JP. Immune microenvironment in tumor progression: characteristics and challenges for therapy. Journal of oncology 2012: 608406, 2012.
12. Lei X, Lei Y, Li JK, Du WX, Li RG, Yang J, Li J, Li F, and Tan HB. Immune cells within the tumor microenvironment: Biological functions and roles in cancer immunotherapy. Cancer letters 470: 126-133, 2020.
13. Viel S, Vivier E, Walzer T, and Marçais A. Targeting metabolic dysfunction of CD8 T cells and natural killer cells in cancer. Nature Reviews Drug Discovery 2024.
14. Kao KC, Vilbois S, Tsai CH, and Ho PC. Metabolic communication in the tumour-immune microenvironment. Nature cell biology 24: 1574-1583, 2022.
15. Bader JE, Voss K, and Rathmell JC. Targeting Metabolism to Improve the Tumor Microenvironment for Cancer Immunotherapy. Molecular cell 78: 1019-1033, 2020.
16. He A, Pu Y, Jia C, Wu M, He H, and Xia Y. The Influence of Exercise on Cancer Risk, the Tumor Microenvironment and the Treatment of Cancer. Sports medicine (Auckland, NZ) 2024.
17. Hojman P, Gehl J, Christensen JF, and Pedersen BK. Molecular Mechanisms Linking Exercise to Cancer Prevention and Treatment. Cell metabolism 27: 10-21, 2018.
18. Buss LA, and Dachs GU. Effects of Exercise on the Tumour Microenvironment. Advances in experimental medicine and biology 1225: 31-51, 2020.
19. Miao S-N, Chai M-Q, Liu X-Y, Wei C-Y, Zhang C-C, Sun N-N, Fei Q-Z, Peng L-L, and Qiu H. Exercise accelerates recruitment of CD8+ T cell to promotes anti-tumor immunity in lung cancer via epinephrine. BMC cancer 24: 474, 2024.

20. Zhu C, Ma H, He A, Li Y, He C, and Xia Y. Exercise in cancer prevention and anticancer therapy: Efficacy, molecular mechanisms and clinical information. Cancer letters 544: 215814, 2022.
21. Sparks LM. Exercise training response heterogeneity: physiological and molecular insights. Diabetologia 60: 2329-2336, 2017.
22. Wang Z, Wang Z, Chen H, Li S, Yang J, Ma Y, Zhou C, Jin X, Liu J, and Wang X. Exercise therapy: Anti-tumor and improving chemotherapy efficacy. Journal of Holistic Integrative Pharmacy 5: 185-194, 2024.
23. Neufer PD, Bamman Marcas M, Muoio Deborah M, Bouchard C, Cooper Dan M, Goodpaster Bret H, Booth Frank W, Kohrt Wendy M, Gerszten Robert E, Mattson Mark P, Hepple Russell T, Kraus William E, Reid Michael B, Bodine Sue C, Jakicic John M, Fleg Jerome L, Williams John P, Joseph L, Evans M, Maruvada P, Rodgers M, Roary M, Boyce Amanda T, Drugan Jonelle K, Koenig James I, Ingraham Richard H, Krotoski D, Garcia-Cazarin M, McGowan Joan A, and Laughlin Maren R. Understanding the Cellular and Molecular Mechanisms of Physical Activity-Induced Health Benefits. Cell metabolism 22: 4-11, 2015.
24. Warburg O. On the origin of cancer cells. Science (New York, NY) 123: 309-314, 1956.
25. Hönigova K, Navratil J, Peltanova B, Polanska HH, Raudenska M, and Masarik M. Metabolic tricks of cancer cells. Biochimica et biophysica acta Reviews on cancer 1877: 188705, 2022.
26. Liao M, Yao D, Wu L, Luo C, Wang Z, Zhang J, and Liu B. Targeting the Warburg effect: A revisited perspective from molecular mechanisms to traditional and innovative therapeutic strategies in cancer. Acta pharmaceutica Sinica B 14: 953-1008, 2024.
27. Ni X, Lu CP, Xu GQ, and Ma JJ. Transcriptional regulation and post-translational modifications in the glycolytic pathway for targeted cancer therapy. Acta pharmacologica Sinica 2024.
28. Du A, Wang Z, Huang T, Xue S, Jiang C, Qiu G, and Yuan K. Fatty acids in cancer: Metabolic functions and potential treatment. 2: e25, 2023.
29. Du D, Liu C, Qin M, Zhang X, Xi T, Yuan S, Hao H, and Xiong J. Metabolic dysregulation and emerging therapeutical targets for hepatocellular carcinoma. Acta pharmaceutica Sinica B 12: 558-580, 2022.
30. Bergers G, and Fendt SM. The metabolism of cancer cells during metastasis. Nature reviews Cancer 21: 162-180, 2021.
31. Arner EN, and Rathmell JC. Metabolic programming and immune suppression in the tumor microenvironment. Cancer cell 41: 421-433, 2023.
32. Bejarano L, Jordão MJC, and Joyce JA. Therapeutic Targeting of the Tumor Microenvironment. Cancer discovery 11: 933-959, 2021.
33. Kroemer G, and Pouyssegur J. Tumor cell metabolism: cancer's Achilles' heel. Cancer cell 13: 472-482, 2008.
34. De Martino M, Rathmell JC, Galluzzi L, and Vanpouille-Box C. Cancer cell metabolism and antitumour immunity. Nature reviews Immunology 24: 654-669, 2024.
35. Liu Y, Wang F, Peng D, Zhang D, Liu L, Wei J, Yuan J, Zhao L, Jiang H, Zhang T, Li Y, Zhao C, He S, Wu J, Yan Y, Zhang P, Guo C, Zhang J, Li X, Gao H, and Li K. Activation and antitumor immunity of CD8(+) T cells are supported by the glucose transporter GLUT10 and disrupted by lactic acid. Science translational medicine 16: eadk7399, 2024.
36. Chen T, Xu ZG, Luo J, Manne RK, Wang Z, Hsu CC, Pan BS, Cai Z, Tsai PJ, Tsai YS, Chen ZZ, Li HY, and Lin HK. NSUN2 is a glucose sensor suppressing cGAS/STING to maintain tumorigenesis and immunotherapy resistance. Cell metabolism 35: 1782-1798.e1788, 2023.
37. De Leo A, Ugolini A, Yu X, Scirocchi F, Scocozza D, Peixoto B, Pace A, D'Angelo L, Liu JKC, Etame AB, Rughetti A, Nuti M, Santoro A, Vogelbaum MA, Conejo-Garcia JR, Rodriguez PC, and Veglia F. Glucose-driven histone lactylation promotes the immunosuppressive activity of monocyte-derived macrophages in glioblastoma. Immunity 57: 1105-1123. e1108, 2024.
38. Duan Q, Li H, Gao C, Zhao H, Wu S, Wu H, Wang C, Shen Q, and Yin T. High glucose promotes pancreatic cancer cells to escape from immune surveillance via AMPK-Bmi1-GATA2-MICA/B pathway. Journal of experimental & clinical cancer research : CR 38: 192, 2019.
39. Wu L, Jin Y, Zhao X, Tang K, Zhao Y, Tong L, Yu X, Xiong K, Luo C, Zhu J, Wang F, Zeng Z, and Pan D. Tumor aerobic glycolysis confers immune evasion through modulating sensitivity to T cell-mediated bystander killing via TNF-α. Cell metabolism 35: 1580-1596.e1589, 2023.
40. Hu Z, Yu X, Ding R, Liu B, Gu C, Pan XW, Han Q, Zhang Y, Wan J, Cui XG, Sun J, and Zou Q. Glycolysis drives STING signaling to facilitate dendritic cell antitumor function. The Journal of clinical investigation 133: 2023.
41. Sen K, Pati R, Jha A, Mishra GP, Prusty S, Chaudhary S, Swetalika S, Podder S, Sen A, Swain M, Nanda RK, and Raghav SK. NCoR1 controls immune tolerance in conventional dendritic cells by fine-tuning glycolysis and fatty acid oxidation. Redox biology 59: 102575, 2023.
42. Xiao G, Chan LN, Klemm L, Braas D, Chen Z, Geng H, Zhang QC, Aghajanirefah A, Cosgun KN, Sadras T, Lee J, Mirzapoiazova T, Salgia R, Ernst T, Hochhaus A, Jumaa H, Jiang X, Weinstock DM, Graeber TG, and Müschen M. B-Cell-Specific Diversion of Glucose Carbon Utilization Reveals a Unique Vulnerability in B Cell Malignancies. Cell 173: 470-484.e418, 2018.
43. Ehm PAH, Horn S, Hoffer K, Kriegs M, Horn M, Giehler S, Nalaskowski M, Rehbach C, Horstmann MA, and Jücker M. Ikaros sets the threshold for negative B-cell selection by regulation of the signaling strength of the AKT pathway. Cell communication and signaling : CCS 22: 360, 2024.
44. Kumagai S, Koyama S, Itahashi K, Tanegashima T, Lin YT, Togashi Y, Kamada T, Irie T, Okumura G, Kono H, Ito D, Fujii R, Watanabe S, Sai A, Fukuoka S, Sugiyama E, Watanabe G, Owari T, Nishinakamura H, Sugiyama D, Maeda Y, Kawazoe A, Yukami H, Chida K, Ohara Y, Yoshida T, Shinno Y, Takeyasu Y, Shirasawa M, Nakama K, Aokage K, Suzuki J, Ishii G, Kuwata T, Sakamoto N, Kawazu M, Ueno T, Mori T, Yamazaki N, Tsuboi M, Yatabe Y, Kinoshita T, Doi T, Shitara K, Mano H, and Nishikawa H. Lactic acid promotes PD-1 expression in regulatory T cells in highly glycolytic tumor microenvironments. Cancer cell 40: 201-218.e209, 2022.
45. Imahashi N, Basar R, Huang Y, Wang F, Baran N, Banerjee PP, Lu J, Nunez Cortes AK, Uprety N, Ensley E, Muniz-Feliciano L, Laskowski TJ, Moyes JS, Daher M, Mendt M, Kerbauy LN, Shanley M, Li L, Lim F, Shaim H, Li Y, Konopleva M, Green M, Wargo J, Shpall EJ, Chen K, and Rezvani K. Activated B cells suppress T-cell function through metabolic competition. Journal for immunotherapy of cancer 10: 2022.
46. Liu X, Wang X, Zhang J, Tian T, Ning Y, Chen Y, Li G, and Cui Z. Myc-mediated inhibition of HIF1a degradation promotes M2 macrophage polarization and impairs CD8 T cell function through lactic acid secretion in ovarian cancer. International immunopharmacology 141: 112876, 2024.

47. Juras PK, Racioppi L, Mukherjee D, Artham S, Gao X, Akullian D'Agostino L, Chang CY, and McDonnell DP. Increased CaMKK2 Expression Is an Adaptive Response That Maintains the Fitness of Tumor-Infiltrating Natural Killer Cells. Cancer immunology research 11: 109-122, 2023.
48. Harmon C, Robinson MW, Hand F, Almuaili D, Mentor K, Houlihan DD, Hoti E, Lynch L, Geoghegan J, and O'Farrelly C. Lactate-Mediated Acidification of Tumor Microenvironment Induces Apoptosis of Liver-Resident NK Cells in Colorectal Liver Metastasis. Cancer immunology research 7: 335-346, 2019.
49. Plebanek MP, Xue Y, Nguyen YV, DeVito NC, Wang X, Holtzhausen A, Beasley GM, Theivanthiran B, and Hanks BA. A lactate-SREBP2 signaling axis drives tolerogenic dendritic cell maturation and promotes cancer progression. Science immunology 9: eadi4191, 2024.
50. Babl N, Decking SM, Voll F, Althammer M, Sala-Hojman A, Ferretti R, Korf C, Schmidl C, Schmidleithner L, Nerb B, Matos C, Koehl GE, Siska P, Bruss C, Kellermeier F, Dettmer K, Oefner PJ, Wichland M, Ugele I, Bohr C, Herr W, Ramaswamy S, Heinrich T, Herhaus C, Kreutz M, and Renner K. MCT4 blockade increases the efficacy of immune checkpoint blockade. Journal for immunotherapy of cancer 11: 2023.
51. Peralta RM, Xie B, Lontos K, Nieves-Rosado H, Spahr K, Joshi S, Ford BR, Quann K, Frisch AT, Dean V, Philbin M, Cillo AR, Gingras S, Poholek AC, Kane LP, Rivadeneira DB, and Delgoffe GM. Dysfunction of exhausted T cells is enforced by MCT11-mediated lactate metabolism. Nature immunology 2024.
52. Feng Q, Liu Z, Yu X, Huang T, Chen J, Wang J, Wilhelm J, Li S, Song J, Li W, Sun Z, Sumer BD, Li B, Fu YX, and Gao J. Lactate increases stemness of CD8+T cells to augment anti-tumor immunity. Nature communications 13: 4981, 2022.
53. Han S, Bao X, Zou Y, Wang L, Li Y, Yang L, Liao A, Zhang X, Jiang X, Liang D, Dai Y, Zheng QC, Yu Z, and Guo J. d-lactate modulates M2 tumor-associated macrophages and remodels immunosuppressive tumor microenvironment for hepatocellular carcinoma. Science advances 9: eadg2697, 2023.
54. Gnanaprakasam JNR, Kushwaha B, Liu L, Chen X, Kang S, Wang T, Cassel TA, Adams CM, Higashi RM, Scott DA, Xin G, Li Z, Yang J, Lane AN, Fan TW, Zhang J, and Wang R. Asparagine restriction enhances CD8(+) T cell metabolic fitness and antitumoral functionality through an NRF2-dependent stress response. Nature metabolism 5: 1423-1439, 2023.
55. Ta HM, Roy D, Zhang K, Alban T, Juric I, Dong J, Parthasarathy PB, Patnaik S, Delaney E, Gilmour C, Zakeri A, Shukla N, Rupani A, Phoon YP, Liu C, Avril S, Gastman B, Chan T, and Wang LL. LRIG1 engages ligand VISTA and impairs tumor-specific CD8(+) T cell responses. Science immunology 9: eadi7418, 2024.
56. Marijt KA, Griffioen L, Blijleven L, van der Burg SH, and van Hall T. Cross-presentation of a TAP-independent signal peptide induces CD8 T immunity to escaped cancers but necessitates anchor replacement. Cancer immunology, immunotherapy : CII 71: 289-300, 2022.
57. Yang C, Wu S, Mou Z, Zhou Q, Dai X, Ou Y, Chen X, Chen Y, Xu C, Hu Y, Zhang L, Zou L, Jin S, Hu J, Mao S, and Jiang H. Exosome-derived circTRPS1 promotes malignant phenotype and CD8+ T cell exhaustion in bladder cancer microenvironments. Molecular therapy : the journal of the American Society of Gene Therapy 30: 1054-1070, 2022.
58. Guo C, You Z, Shi H, Sun Y, Du X, Palacios G, Guy C, Yuan S, Chapman NM, Lim SA, Sun X, Saravia J, Rankin S, Dhungana Y, and Chi H. SLC38A2 and glutamine signalling in cDC1s dictate anti-tumour immunity. Nature 620: 200-208, 2023.
59. Li LB, Fang TY, and Xu WJ. Oral glutamine inhibits tumor growth of gastric cancer bearing mice by improving immune function and activating apoptosis pathway. Tissue & cell 71: 101508, 2021.
60. Siska PJ, Jiao J, Matos C, Singer K, Berger RS, Dettmer K, Oefner PJ, Cully MD, Wang Z, Quinn IW, Oliff KN, Wilkins BJ, Christensen LM, Wang L, Hancock WW, Baur JA, Levine MH, Ugele I, Mayr R, Renner K, Zhou L, Kreutz M, and Beier UH. Kynurenine induces T cell fat catabolism and has limited suppressive effects in vivo. EBioMedicine 74: 103734, 2021.
61. Bender MJ, McPherson AC, Phelps CM, Pandey SP, Laughlin CR, Shapira JH, Medina Sanchez L, Rana M, Richie TG, Mims TS, Gocher-Demske AM, Cervantes-Barragan L, Mullett SJ, Gelhaus SL, Bruno TC, Cannon N, McCulloch JA, Vignali DAA, Hinterleitner R, Joglekar AV, Pierre JF, Lee STM, Davar D, Zarour HM, and Meisel M. Dietary tryptophan metabolite released by intratumoral Lactobacillus reuteri facilitates immune checkpoint inhibitor treatment. Cell 186: 1846-1862.e1826, 2023.
62. Kurniawan H, Franchina DG, Guerra L, Bonetti L, Baguet LS, Grusdat M, Schlicker L, Hunewald O, Dostert C, Merz MP, Binsfeld C, Duncan GS, Farinelle S, Nonnenmacher Y, Haight J, Das Gupta D, Ewen A, Taskesen R, Halder R, Chen Y, Jäger C, Ollert M, Wilmes P, Vasiliou V, Harris IS, Knobbe-Thomsen CB, Turner JD, Mak TW, Lohoff M, Meiser J, Hiller K, and Brenner D. Glutathione Restricts Serine Metabolism to Preserve Regulatory T Cell Function. Cell metabolism 31: 920-936.e927, 2020.
63. Tharp KM, Kersten K, Maller O, Timblin GA, Stashko C, Canale FP, Menjivar RE, Hayward MK, Berestjuk I, Ten Hoeve J, Samad B, Ironside AJ, di Magliano MP, Muir A, Geiger R, Combes AJ, and Weaver VM. Tumor-associated macrophages restrict CD8(+) T cell function through collagen deposition and metabolic reprogramming of the breast cancer microenvironment. Nature cancer 5: 1045-1062, 2024.
64. Kumagai S, Togashi Y, Sakai C, Kawazoe A, Kawazu M, Ueno T, Sato E, Kuwata T, Kinoshita T, Yamamoto M, Nomura S, Tsukamoto T, Mano H, Shitara K, and Nishikawa H. An Oncogenic Alteration Creates a Microenvironment that Promotes Tumor Progression by Conferring a Metabolic Advantage to Regulatory T Cells. Immunity 53: 187-203.e188, 2020.
65. Liu Z, Liu W, Wang W, Ma Y, Wang Y, Drum DL, Cai J, Blevins H, Lee E, Shah S, Fisher PB, Wang X, Fang X, Guo C, and Wang XY. CPT1A-mediated fatty acid oxidation confers cancer cell resistance to immune-mediated cytolytic killing. Proceedings of the National Academy of Sciences of the United States of America 120: e2302878120, 2023.
66. Li Z, Wang Y, Xing R, Zeng H, Yu XJ, Zhang YJ, Xu J, and Zheng L. Cholesterol Efflux Drives the Generation of Immunosuppressive Macrophages to Promote the Progression of Human Hepatocellular Carcinoma. Cancer immunology research 11: 1400-1413, 2023.
67. Liao P, Wang W, Wang W, Kryczek I, Li X, Bian Y, Sell A, Wei S, Grove S, Johnson JK, Kennedy PD, Gijón M, Shah YM, and Zou W. CD8(+) T cells and fatty acids orchestrate tumor ferroptosis and immunity via ACSL4. Cancer cell 40: 365-378.e366, 2022.
68. Zhu L, Zhu X, and Wu Y. Effects of Glucose Metabolism, Lipid Metabolism, and Glutamine Metabolism on Tumor Microenvironment and Clinical Implications. Biomolecules 12: 2022.

69. Enzo E, Santinon G, Pocaterra A, Aragona M, Bresolin S, Forcato M, Grifoni D, Pession A, Zanconato F, Guzzo G, Bicciato S, and Dupont S. Aerobic glycolysis tunes YAP/TAZ transcriptional activity. The EMBO journal 34: 1349-1370, 2015.
70. Shi Q, Shen Q, Liu Y, Shi Y, Huang W, Wang X, Li Z, Chai Y, Wang H, Hu X, Li N, Zhang Q, and Cao X. Increased glucose metabolism in TAMs fuels O-GlcNAcylation of lysosomal Cathepsin B to promote cancer metastasis and chemoresistance. Cancer cell 40: 1207-1222.e1210, 2022.
71. Notarangelo G, Spinelli JB, Perez EM, Baker GJ, Kurmi K, Elia I, Stopka SA, Baquer G, Lin JR, Golby AJ, Joshi S, Baron HF, Drijvers JM, Georgiev P, Ringel AE, Zaganjor E, McBrayer SK, Sorger PK, Sharpe AH, Wucherpfennig KW, Santagata S, Agar NYR, Suvà ML, and Haigis MC. Oncometabolite d-2HG alters T cell metabolism to impair CD8(+) T cell function. Science (New York, NY) 377: 1519-1529, 2022.
72. Wang T, Ye Z, Li Z, Jing DS, Fan GX, Liu MQ, Zhuo QF, Ji SR, Yu XJ, Xu XW, and Qin Y. Lactate-induced protein lactylation: A bridge between epigenetics and metabolic reprogramming in cancer. Cell proliferation 56: e13478, 2023.
73. Chen S, Xu Y, Zhuo W, and Zhang L. The emerging role of lactate in tumor microenvironment and its clinical relevance. Cancer letters 590: 216837, 2024.
74. Jia M, Yue X, Sun W, Zhou Q, Chang C, Gong W, Feng J, Li X, Zhan R, Mo K, Zhang L, Qian Y, Sun Y, Wang A, Zou Y, Chen W, Li Y, Huang L, Yang Y, Zhao Y, and Cheng X. ULK1-mediated metabolic reprogramming regulates Vps34 lipid kinase activity by its lactylation. Science advances 9: eadg4993, 2023.
75. Oseguera M, and Yau AA. Lactic acidosis in a patient with cancer. Clinical medicine (London, England) 23: 615-617, 2023.
76. Groessl S, Kalis R, Snaebjornsson MT, Wambach L, Haider J, Andersch F, Schulze A, Palm W, and Zuber J. Acidosis orchestrates adaptations of energy metabolism in tumors. Science (New York, NY) 390: eadp7603, 2025.
77. Apostolova P, and Pearce EL. Lactic acid and lactate: revisiting the physiological roles in the tumor microenvironment. Trends in immunology 43: 969-977, 2022.
78. Wen J, Cheng S, Zhang Y, Wang R, Xu J, Ling Z, Ma L, Ai X, and Sun B. Lactate anions participate in T cell cytokine production and function. Science China Life sciences 64: 1895-1905, 2021.
79. Rundqvist H, Veliça P, Barbieri L, Gameiro PA, Bargiela D, Gojkovic M, Mijwel S, Reitzner SM, Wulliman D, Ahlstedt E, Ule J, Östman A, and Johnson RS. Cytotoxic T-cells mediate exercise-induced reductions in tumor growth. eLife 9: e59996, 2020.
80. Chen J, Cui L, Lu S, and Xu S. Amino acid metabolism in tumor biology and therapy. Cell death & disease 15: 42, 2024.
81. Lieu EL, Nguyen T, Rhyne S, and Kim J. Amino acids in cancer. Experimental & molecular medicine 52: 15-30, 2020.
82. Zhang J, Pavlova NN, and Thompson CB. Cancer cell metabolism: the essential role of the nonessential amino acid, glutamine. The EMBO journal 36: 1302-1315, 2017.
83. Yoo HC, Park SJ, Nam M, Kang J, Kim K, Yeo JH, Kim JK, Heo Y, Lee HS, Lee MY, Lee CW, Kang JS, Kim YH, Lee J, Choi J, Hwang GS, Bang S, and Han JM. A Variant of SLC1A5 Is a Mitochondrial Glutamine Transporter for Metabolic Reprogramming in Cancer Cells. Cell metabolism 31: 267-283.e212, 2020.
84. Mossmann D, Müller C, Park S, Ryback B, Colombi M, Ritter N, Weißenberger D, Dazert E, Coto-Llerena M, Nuciforo S, Blukacz L, Ercan C, Jimenez V, Piscuoglio S, Bosch F, Terracciano LM, Sauer U, Heim MH, and Hall MN. Arginine reprograms metabolism in liver cancer via RBM39. Cell 186: 5068-5083. e5023, 2023.
85. Martin-Perez M, Urdiroz-Urricelqui U, Bigas C, and Benitah SA. The role of lipids in cancer progression and metastasis. Cell metabolism 34: 1675-1699, 2022.
86. Broadfield LA, Pane AA, Talebi A, Swinnen JV, and Fendt SM. Lipid metabolism in cancer: New perspectives and emerging mechanisms. Developmental cell 56: 1363-1393, 2021.
87. Geng F, and Guo D. SREBF1/SREBP-1 concurrently regulates lipid synthesis and lipophagy to maintain lipid homeostasis and tumor growth. Autophagy 20: 1183-1185, 2024.
88. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. Nature Reviews Immunology 5: 375-386, 2005.
89. Li SB, Yuan L, Zhu QY, Zhang G, Liu YT, Gao MH, Han F, Lin ZR, Zhang H, Tang LQ, Sun L, He QY, Song EW, Young LS, Li YC, Mai HQ, Zeng MS, and Zhong Q. OASL enhances mRNA translation and reprograms lipid metabolism to promote cancer progression. Cell reports 44: 115901, 2025.
90. Zhou H, Wang W, Xu H, Liang Y, Ding J, Lv M, Ren B, Peng H, Fu YX, and Zhu M. Metabolic reprograming mediated by tumor cell-intrinsic type I IFN signaling is required for CD47-SIRPα blockade efficacy. Nature communications 15: 5759, 2024.
91. Hu B, Yu M, Ma X, Sun J, Liu C, Wang C, Wu S, Fu P, Yang Z, He Y, Zhu Y, Huang C, Yang X, Shi Y, Qiu S, Sun H, Zhu AX, Zhou J, Xu Y, Zhu D, and Fan J. IFNα Potentiates Anti-PD-1 Efficacy by Remodeling Glucose Metabolism in the Hepatocellular Carcinoma Microenvironment. Cancer discovery 12: 1718-1741, 2022.
92. Tsai CH, Chuang YM, Li X, Yu YR, Tzeng SF, Teoh ST, Lindblad KE, Di Matteo M, Cheng WC, Hsueh PC, Kao KC, Imrichova H, Duan L, Gallart-Ayala H, Hsiao PW, Mazzone M, Ivanesevic J, Liu X, de Visser KE, Lujambio A, Lunt SY, Kaech SM, and Ho PC. Immunoediting instructs tumor metabolic reprogramming to support immune evasion. Cell metabolism 35: 118-133.e117, 2023.
93. Li G, Choi JE, Kryczek I, Sun Y, Liao P, Li S, Wei S, Grove S, Vatan L, Nelson R, Schaefer G, Allen SG, Sankar K, Fecher LA, Mendiratta-Lala M, Frankel TL, Qin A, Waninger JJ, Tezel A, Alva A, Lao CD, Ramnath N, Cieslik M, Harms PW, Green MD, Chinnaiyan AM, and Zou W. Intersection of immune and oncometabolic pathways drives cancer hyperprogression during immunotherapy. Cancer cell 41: 304-322.e307, 2023.
94. Shinde A, Chandak N, Singh J, Roy M, Mane M, Tang X, Vasiyani H, Currim F, Gohel D, Shukla S, Goyani S, Saranga MV, Brindley DN, and Singh R. TNF-α induced NF-κB mediated LYRM7 expression modulates the tumor growth and metastatic ability in breast cancer. Free Radical Biology and Medicine 211: 158-170, 2024.
95. Jeong H, Kim S, Hong BJ, Lee CJ, Kim YE, Bok S, Oh JM, Gwak SH, Yoo MY, Lee MS, Chung SJ, Defrêne J, Tessier P, Pelletier M, Jeon H, Roh TY, Kim B, Kim KH, Ju JH, Kim S, Lee YJ, Kim DW, Kim IH, Kim HJ, Park JW, Lee YS, Lee JS, Cheon GJ, Weissman IL, Chung DH, Jeon YK, and Ahn GO. Tumor-Associated Macrophages Enhance Tumor Hypoxia and Aerobic Glycolysis. Cancer research 79: 795-806, 2019.
96. Taranto D, Kloosterman DJ, and Akkari L. Macrophages and T cells in metabolic disorder-associated cancers. Nature reviews Cancer 24: 744-767, 2024.
97. Goldman O, Adler LN, Hajaj E, Croese T, Darzi N, Galai S, Tishler H, Ariav Y, Lavie D, Fellus-Alyagor L, Oren R, Kuznetsov Y, David E, Jaschek R, Stossel C, Singer O, Malitsky S, Barak R, Seger R, Erez N, Amit I, Tanay A, Saada A, Golan T, Rubinek T, Sang Lee J, Ben-Shachar S, Wolf I, and Erez A. Early Infiltration of Innate Immune Cells to the Liver Depletes HNF4α and Promotes Extrahepatic Carcinogenesis. Cancer discovery 13: 1616-1635, 2023.

98. van der Windt GJ, Everts B, Chang CH, Curtis JD, Freitas TC, Amiel E, Pearce EJ, and Pearce EL. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity 36: 68-78, 2012.
99. Buck MD, O'Sullivan D, Klein Geltink RI, Curtis JD, Chang CH, Sanin DE, Qiu J, Kretz O, Braas D, van der Windt GJ, Chen Q, Huang SC, O'Neill CM, Edelson BT, Pearce EJ, Sesaki H, Huber TB, Rambold AS, and Pearce EL. Mitochondrial Dynamics Controls T Cell Fate through Metabolic Programming. Cell 166: 63-76, 2016.
100. Marçais A, Cherfils-Vicini J, Viant C, Degouve S, Viel S, Fenis A, Rabilloud J, Mayol K, Tavares A, Bienvenu J, Gangloff YG, Gilson E, Vivier E, and Walzer T. The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. Nature immunology 15: 749-757, 2014.
101. Felices M, Lenvik AJ, McElmurry R, Chu S, Hinderlie P, Bendzick L, Geller MA, Tolar J, Blazar BR, and Miller JS. Continuous treatment with IL-15 exhausts human NK cells via a metabolic defect. JCI insight 3: 2018.
102. Alizadeh D, Wong RA, Yang X, Wang D, Pecoraro JR, Kuo CF, Aguilar B, Qi Y, Ann DK, Starr R, Urak R, Wang X, Forman SJ, and Brown CE. IL15 Enhances CAR-T Cell Antitumor Activity by Reducing mTORC1 Activity and Preserving Their Stem Cell Memory Phenotype. Cancer immunology research 7: 759-772, 2019.
103. Matthews CE, Moore SC, Arem H, Cook MB, Trabert B, Håkansson N, Larsson SC, Wolk A, Gapstur SM, Lynch BM, Milne RL, Freedman ND, Huang WY, Berrington de Gonzalez A, Kitahara CM, Linet MS, Shiroma EJ, Sandin S, Patel AV, and Lee IM. Amount and Intensity of Leisure-Time Physical Activity and Lower Cancer Risk. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 38: 686-697, 2020.
104. Moore SC, Lee IM, Weiderpass E, Campbell PT, Sampson JN, Kitahara CM, Keadle SK, Arem H, Berrington de Gonzalez A, Hartge P, Adami HO, Blair CK, Borch KB, Boyd E, Check DP, Fournier A, Freedman ND, Gunter M, Johannson M, Khaw KT, Linet MS, Orsini N, Park Y, Riboli E, Robien K, Schairer C, Sesso H, Spriggs M, Van Dusen R, Wolk A, Matthews CE, and Patel AV. Association of Leisure-Time Physical Activity With Risk of 26 Types of Cancer in 1.44 Million Adults. JAMA internal medicine 176: 816-825, 2016.
105. Torregrosa C, Chorin F, Beltran EEM, Neuzillet C, and Cardot-Ruffino V. Physical Activity as the Best Supportive Care in Cancer: The Clinician's and the Researcher's Perspectives. Cancers 14: 2022.
106. Koelwyn GJ, Zhuang X, Tammela T, Schietinger A, and Jones LW. Exercise and immunometabolic regulation in cancer. Nature metabolism 2: 849-857, 2020.
107. Sheinboim D, Parikh S, Manich P, Markus I, Dahan S, Parikh R, Stubbs E, Cohen G, Zemser-Werner V, Bell RE, Ruiz SA, Percik R, Brenner R, Leibou S, Vaknine H, Arad G, Gerber Y, Keinan-Boker L, Shimony T, Bikovski L, Goldstein N, Constantini K, Labes S, Mordechai S, Doron H, Lonescu A, Ziv T, Nizri E, Choshen G, Eldar-Finkelman H, Tabach Y, Helman A, Ben-Eliyahu S, Erez N, Perlson E, Geiger T, Ben-Zvi D, Khaled M, Gepner Y, and Levy C. An Exercise-Induced Metabolic Shield in Distant Organs Blocks Cancer Progression and Metastatic Dissemination. Cancer research 82: 4164-4178, 2022.
108. Mika A, Macaluso F, Barone R, Di Felice V, and Sledzinski T. Effect of Exercise on Fatty Acid Metabolism and Adipokine Secretion in Adipose Tissue. Frontiers in physiology 10: 26, 2019.
109. Aveseh M, Nikooie R, and Aminaie M. Exercise-induced changes in tumour LDH-B and MCT1 expression are modulated by oestrogen-related receptor alpha in breast cancer-bearing BALB/c mice. The Journal of physiology 593: 2635-2648, 2015.
110. Davis AR, Goodenough CG, Westerlind KC, Strange R, Deaver JW, Ryan PJ, Riechman SE, and Fluckey JD. Myokines derived from contracting skeletal muscle suppress anabolism in MCF7 breast cancer cells by inhibiting mTOR. Frontiers in physiology 13: 1033585, 2022.
111. Huang Q, Wu M, Wu X, Zhang Y, and Xia Y. Muscle-to-tumor crosstalk: The effect of exercise-induced myokine on cancer progression. Biochimica et biophysica acta Reviews on cancer 1877: 188761, 2022.
112. Nieman DC, and Pence BD. Exercise immunology: Future directions. Journal of sport and health science 9: 432-445, 2020.
113. Mohr AE, Jäger R, Carpenter KC, Kerksick CM, Purpura M, Townsend JR, West NP, Black K, Gleeson M, Pyne DB, Wells SD, Arent SM, Kreider RB, Campbell BI, Bannock L, Scheiman J, Wissent CJ, Pane M, Kalman DS, Pugh JN, Ortega-Santos CP, Ter Haar JA, Arciero PJ, and Antonio J. The athletic gut microbiota. Journal of the International Society of Sports Nutrition 17: 24, 2020.
114. Smith JAB, Murach KA, Dyar KA, and Zierath JR. Exercise metabolism and adaptation in skeletal muscle. Nature reviews Molecular cell biology 24: 607-632, 2023.
115. Egan B, and Zierath JR. Exercise metabolism and the molecular regulation of skeletal muscle adaptation. Cell metabolism 17: 162-184, 2013.
116. Isath A, Koziol KJ, Martinez MW, Garber CE, Martinez MN, Emery MS, Baggish AL, Naidu SS, Lavie CJ, Arena R, and Krittanawong C. Exercise and cardiovascular health: A state-of-the-art review. Progress in cardiovascular diseases 79: 44-52, 2023.
117. Chen H, Chen C, Spanos M, Li G, Lu R, Bei Y, and Xiao J. Exercise training maintains cardiovascular health: signaling pathways involved and potential therapeutics. Signal transduction and targeted therapy 7: 306, 2022.
118. Powers SK, Sollanek KJ, Wiggs MP, Demirel HA, and Smuder AJ. Exercise-induced improvements in myocardial antioxidant capacity: the antioxidant players and cardioprotection. Free radical research 48: 43-51, 2014.
119. Gao Y, Zhang W, Zeng LQ, Bai H, Li J, Zhou J, Zhou GY, Fang CW, Wang F, and Qin XJ. Exercise and dietary intervention ameliorate high-fat diet-induced NAFLD and liver aging by inducing lipophagy. Redox biology 36: 101635, 2020.
120. Thyfault JP, and Bergouignan A. Exercise and metabolic health: beyond skeletal muscle. Diabetologia 63: 1464-1474, 2020.
121. Keating SE, Sabag A, Hallsworth K, Hickman IJ, Macdonald GA, Stine JG, George J, and Johnson NA. Exercise in the Management of Metabolic-Associated Fatty Liver Disease (MAFLD) in Adults: A Position Statement from Exercise and Sport Science Australia. Sports medicine (Auckland, NZ) 53: 2347-2371, 2023.
122. Kuramoto K, Liang H, Hong JH, and He C. Exercise-activated hepatic autophagy via the FN1-α5β1 integrin pathway drives metabolic benefits of exercise. Cell metabolism 35: 620-632. e625, 2023.
123. Clark A, and Mach N. Exercise-induced stress behavior, gut-microbiota-brain axis and diet: a systematic review for athletes. Journal of the International Society of Sports Nutrition 13: 43, 2016.
124. Markandey M, Bajaj A, Ilott NE, Kedia S, Travis S, Powrie F, and Ahuja V. Gut microbiota: sculptors of the intestinal stem cell niche in health and inflammatory bowel disease. Gut microbes 13: 1990827, 2021.

125. Gubert C, Kong G, Renoir T, and Hannan AJ. Exercise, diet and stress as modulators of gut microbiota: Implications for neurodegenerative diseases. Neurobiology of disease 134: 104621, 2020.
126. Luo Z, Mei J, Wang X, Wang R, He Z, Geffen Y, Sun X, Zhang X, Xu J, Wan R, Feng X, Jiao C, Su X, Sun J, Chen S, Chen J, Mao W, Yang Y, and Sun Y. Voluntary exercise sensitizes cancer immunotherapy via the collagen inhibition-orchestrated inflammatory tumor immune microenvironment. Cell reports 43: 114697, 2024.
127. Pedersen L, Idorn M, Olofsson GH, Lauenborg B, Nookaew I, Hansen RH, Johannesen HH, Becker JC, Pedersen KS, Dethlefsen C, Nielsen J, Gehl J, Pedersen BK, Thor Straten P, and Hojman P. Voluntary Running Suppresses Tumor Growth through Epinephrine- and IL-6-Dependent NK Cell Mobilization and Redistribution. Cell metabolism 23: 554-562, 2016.
128. Bacurau RF, Belmonte MA, Seelaender MC, and Costa Rosa LF. Effect of a moderate intensity exercise training protocol on the metabolism of macrophages and lymphocytes of tumour-bearing rats. Cell biochemistry and function 18: 249-258, 2000.
129. Rundqvist H, Veliça P, Barbieri L, Gameiro PA, Bargiela D, Gojkovic M, Mijwel S, Reitzner SM, Wulliman D, Ahlstedt E, Ule J, Östman A, and Johnson RS. Cytotoxic T-cells mediate exercise-induced reductions in tumor growth. eLife 9: 2020.
130. Bacurau AV, Belmonte MA, Navarro F, Moraes MR, Pontes FL, Jr., Pesquero JL, Araújo RC, and Bacurau RF. Effect of a high-intensity exercise training on the metabolism and function of macrophages and lymphocytes of walker 256 tumor bearing rats. Experimental biology and medicine (Maywood, NJ) 232: 1289-1299, 2007.
131. Smith KA, Batatinha H, Niemiro GM, Baker FL, Zúñiga TM, Diak DM, Mylabathula PL, Kistner TM, Davini D, Hoffman E, Colombo JN, Seckeler MD, Bond RA, Katsanis E, and Simpson RJ. Exercise-induced β(2)-adrenergic receptor activation enhances effector lymphocyte mobilization in humans and suppresses lymphoma growth in mice through NK-cells. Brain, behavior, and immunity 128: 751-765, 2025.
132. Batatinha H, Peña NA, Hoskin GA, Kistner TM, Diak DM, Niemiro GM, Katsanis E, and Simpson RJ. Exercise Delays Human Leukemia Progression and Mitigates Graft-Versus-Host Disease After Donor Lymphocyte Infusion in Xenogeneic Mice. Cancers 17: 2025.
133. Kurz E, Hirsch CA, Dalton T, Shadaloey SA, Khodadadi-Jamayran A, Miller G, Pareek S, Rajaei H, Mohindroo C, Baydogan S, Ngo-Huang A, Parker N, Katz MHG, Petzel M, Vucic E, McAllister F, Schadler K, Winograd R, and Bar-Sagi D. Exercise-induced engagement of the IL-15/IL-15Rα axis promotes anti-tumor immunity in pancreatic cancer. Cancer cell 40: 720-737.e725, 2022.
134. Flores-Opazo M, McGee SL, and Hargreaves M. Exercise and GLUT4. Exercise and sport sciences reviews 48: 110-118, 2020.
135. Orange ST, Jordan AR, Odell A, Kavanagh O, Hicks KM, Eaglen T, Todryk S, and Saxton JM. Acute aerobic exercise-conditioned serum reduces colon cancer cell proliferation in vitro through interleukin-6-induced regulation of DNA damage. International journal of cancer 151: 265-274, 2022.
136. Zhu C, Ma H, He A, Li Y, He C, and Xia Y. Exercise in cancer prevention and anticancer therapy: Efficacy, molecular mechanisms and clinical information. Cancer letters 544: 215814, 2022.
137. Piguet AC, Saran U, Simillion C, Keller I, Terracciano L, Reeves HL, and Dufour JF. Regular exercise decreases liver tumors development in hepatocyte-specific PTEN-deficient mice independently of steatosis. Journal of hepatology 62: 1296-1303, 2015.
138. Hojman P, Gehl J, Christensen JF, and Pedersen BK. Molecular Mechanisms Linking Exercise to Cancer Prevention and Treatment. Cell metabolism 27: 10-21, 2018.
139. Savage H, Pareek S, Lee J, Ballarò R, Conterno Minussi D, Hayek K, Sadullozoda M, Lochmann BS, McQuade JL, LaVoy EC, Marmonti E, Patel H, Wang G, Imanishi M, Kotla S, Abe JI, and Schadler K. Aerobic Exercise Alters the Melanoma Microenvironment and Modulates ERK5 S496 Phosphorylation. Cancer immunology research 11: 1168-1183, 2023.
140. Schadler KL, Thomas NJ, Galie PA, Bhang DH, Roby KC, Addai P, Till JE, Sturgeon K, Zaslavsky A, Chen CS, and Ryeom S. Tumor vessel normalization after aerobic exercise enhances chemotherapeutic efficacy. Oncotarget 7: 65429-65440, 2016.
141. Thompson HJ, Jiang W, and Zhu Z. Candidate mechanisms accounting for effects of physical activity on breast carcinogenesis. IUBMB life 61: 895-901, 2009.
142. Theriau CF, Shpilberg Y, Riddell MC, and Connor MK. Voluntary physical activity abolishes the proliferative tumor growth microenvironment created by adipose tissue in animals fed a high fat diet. Journal of applied physiology (Bethesda, Md : 1985) 121: 139-153, 2016.
143. Llavero F, Alejo LB, Fiuza-Luces C, López Soto A, Valenzuela PL, Castillo-García A, Morales JS, Fernández D, Aldazabal IP, Ramírez M, Santos-Lozano A, Zugaza JL, and Lucia A. Exercise training effects on natural killer cells: a preliminary proteomics and systems biology approach. Exercise immunology review 27: 125-141, 2021.
144. de Souza-Teixeira F, Alonso-Molero J, Ayán C, Vilorio-Marques L, Molina AJ, González-Donquiles C, Dávila-Batista V, Fernández-Villa T, de Paz JA, and Martín V. PGC-1α as a Biomarker of Physical Activity-Protective Effect on Colorectal Cancer. Cancer Prevention Research 11: 523-534, 2018.
145. Neto IVS, Pinto AP, Muñoz VR, de Cássia Marqueti R, Pauli JR, Ropelle ER, and Silva A. Pleiotropic and multi-systemic actions of physical exercise on PGC-1α signaling during the aging process. Ageing research reviews 87: 101935, 2023.
146. Bost F, and Kaminski L. The metabolic modulator PGC-1α in cancer. American journal of cancer research 9: 198-211, 2019.
147. Neto IVdS, Pinto AP, Muñoz VR, de Cássia Marqueti R, Pauli JR, Ropelle ER, and Silva ASRd. Pleiotropic and multi-systemic actions of physical exercise on PGC-1α signaling during the aging process. Ageing research reviews 87: 101935, 2023.
148. Memme JM, Erlich AT, Phukan G, and Hood DA. Exercise and mitochondrial health. The Journal of physiology 599: 803-817, 2021.
149. Handschin C, and Spiegelman BM. The role of exercise and PGC1α in inflammation and chronic disease. Nature 454: 463-469, 2008.
150. Li L, Pan R, Li R, Niemann B, Aurich AC, Chen Y, and Rohrbach S. Mitochondrial biogenesis and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) deacetylation by physical activity: intact adipocytokine signaling is required. Diabetes 60: 157-167, 2011.
151. Xia H, Scholtes C, Dufour CR, Guluzian C, and Giguère V. ERRα fosters running endurance by driving myofiber aerobic transformation and fuel efficiency. Molecular metabolism 78: 101814, 2023.

152. Billon C, Sitaula S, Banerjee S, Welch R, Elgendy B, Hegazy L, Oh TG, Kazantzis M, Chatterjee A, Chrivia J, Hayes ME, Xu W, Hamilton A, Huss JM, Zhang L, Walker JK, Downes M, Evans RM, and Burris TP. Synthetic ERRα/β/γ Agonist Induces an ERRα-Dependent Acute Aerobic Exercise Response and Enhances Exercise Capacity. ACS chemical biology 18: 756-771, 2023.
153. Fiuza-Luces C, Valenzuela PL, Gálvez BG, Ramírez M, López-Soto A, Simpson RJ, and Lucia A. The effect of physical exercise on anticancer immunity. Nature Reviews Immunology 24: 282-293, 2024.
154. Li S, Lin J, Huang L, Hu S, Wang M, Sun W, and Sun S. STK31 drives tumor immune evasion through STAT3-IL-6 mediated CD8+ T cell exhaustion. Oncogene 44: 1452-1462, 2025.
155. Tsai SH, Cheng HC, Little JP, Islam H, and Liu HW. Elevated Plasma IL-6 Coincides with Activation of STAT3 in PBMC After Acute Resistance Exercise. Medicine and science in sports and exercise 56: 2117-2124, 2024.
156. Wei L-Y, Lin H-C, Tsai F-C, Ko J-Y, Kok S-H, Cheng S-J, Lee J-J, and Chia J-S. Effects of Interleukin-6 on STAT3-regulated signaling in oral cancer and as a prognosticator of patient survival. Oral Oncology 124: 105665, 2022.
157. Shalamzari SA, Agha-Alinejad H, Alizadeh S, Shahbazi S, Khatib ZK, Kazemi A, Saei MA, and Minayi N. The effect of exercise training on the level of tissue IL-6 and vascular endothelial growth factor in breast cancer bearing mice. Iranian journal of basic medical sciences 17: 231-258, 2014.
158. Fischer CP. Interleukin-6 in acute exercise and training: what is the biological relevance? Exercise immunology review 12: 6-33, 2006.
159. Wiig H, and Swartz MA. Interstitial fluid and lymph formation and transport: physiological regulation and roles in inflammation and cancer. Physiological reviews 92: 1005-1060, 2012.
160. Nash D, Hughes MG, Butcher L, Aicheler R, Smith P, Cullen T, and Webb R. IL-6 signaling in acute exercise and chronic training: Potential consequences for health and athletic performance. Scandinavian journal of medicine & science in sports 33: 4-19, 2023.
161. Campbell KL, Winters-Stone KM, Wiskemann J, May AM, Schwartz AL, Courneya KS, Zucker DS, Matthews CE, Ligibel JA, Gerber LH, Morris GS, Patel AV, Hue TF, Perna FM, and Schmitz KH. Exercise Guidelines for Cancer Survivors: Consensus Statement from International Multidisciplinary Roundtable. Medicine and science in sports and exercise 51: 2375-2390, 2019.
162. Phelps CM, Willis NB, Duan T, Lee AH, Zhang Y, Rodriguez J DM, Pandey SP, Laughlin CR, Rosen ABI, McPherson AC, Shapira JH, Randhawa SK, Hedden L, Richie TG, Wiechman HM, Bender MJ, Nemet I, Zöhrer PA, Gottschalk RA, Schmitz KH, Mullett SJ, Gelhaus SL, Davar D, Zarour HM, Hinterleitner R, Mossington T, Badger JH, Rodrigues RR, McCulloch JA, Lee STM, Wagner K-H, Winter MG, Winter SE, Das J, Pierre JF, Trinchieri G, and Meisel M. Exercise-induced microbiota metabolite enhances CD8 T cell antitumor immunity promoting immunotherapy efficacy. Cell 188: 5680-5700.e5628, 2025.
163. Luo Z, Mei J, Wang X, Wang R, He Z, Geffen Y, Sun X, Zhang X, Xu J, Wan R, Feng X, Jiao C, Su X, Sun J, Chen S, Chen J, Mao W, Yang Y, and Sun Y. Voluntary exercise sensitizes cancer immunotherapy via the collagen inhibition-orchestrated inflammatory tumor immune microenvironment. Cell reports 43: 2024.
164. Clifford B, Koizumi S, Wewege MA, Leake HB, Ha L, Macdonald E, Fairman CM, and Hagstrom AD. The Effect of Resistance Training on Body Composition During and After Cancer Treatment: A Systematic Review and Meta-Analysis. Sports medicine (Auckland, NZ) 51: 2527-2546, 2021.
165. Niu N, Huang R, Zhao J, and Zeng Y. Health benefits of yoga for cancer survivors: An updated systematic review and meta-analysis. Asia-Pacific journal of oncology nursing 11: 100316, 2024.
166. Gomes-Santos IL, Kumar AS, Hausmann F, Meyer MN, Shiferaw SZ, Amoozgar Z, Jain RK, and Fukumura D. Exercise intensity governs tumor control in mice with breast cancer. Frontiers in immunology 15: 1339232, 2024.
167. Bettariga F, Taaffe DR, Galvão DA, Bishop C, Kim JS, and Newton RU. Suppressive effects of exercise-conditioned serum on cancer cells: A narrative review of the influence of exercise mode, volume, and intensity. Journal of sport and health science 13: 484-498, 2024.
168. Isanejad A, Nazari S, Gharib B, and Motlagh AG. Comparison of the effects of high-intensity interval and moderate-intensity continuous training on inflammatory markers, cardiorespiratory fitness, and quality of life in breast cancer patients. Journal of sport and health science 12: 674-689, 2023.
169. Wang J, Song H, Tang X, Yang Y, Vieira VJ, Niu Y, and Ma Y. Effect of exercise training intensity on murine T-regulatory cells and vaccination response. Scandinavian journal of medicine & science in sports 22: 643-652, 2012.
170. Martin RA, Viggars MR, and Esser KA. Metabolism and exercise: the skeletal muscle clock takes centre stage. Nature reviews Endocrinology 19: 272-284, 2023.
171. Wang J, He Y, Wang Z, Wang Z, Miao Y, and Choi JY. Effects of different exercise prescription parameters on metabolic and inflammatory biomarkers in cancer patients: a systematic review, meta-analysis, and meta-regression. Frontiers in immunology 16: 1663560, 2025.
172. Koivula T, Lempiäinen S, Rinne P, Rannikko JH, Hollmén M, Sundberg CJ, Rundqvist H, Minn H, and Heinonen I. The effect of acute exercise on circulating immune cells in newly diagnosed breast cancer patients. Scientific reports 13: 6561, 2023.
173. Amare F, Kiflu A, and Taddese A. Effects of concurrent continuous aerobic and short rest resistance exercise training on metabolic biomarkers in type 2 diabetes patients: a systematic review and meta-analysis. Diabetology & metabolic syndrome 17: 290, 2025.
174. Teodoro TH, Costa KPM, Prestes J, Campanholi J, Navalta J, and Pereira GB. The effects of acute and chronic exercise on immune markers of TH1/TH2 cells in older adults: a systematic review. Frontiers in physiology 16: 1453747, 2025.
175. Zhang N, Wang X, Feng M, Li M, Wang J, Yang H, He S, Xia Z, Shang L, Jiang X, Sun M, Wu Y, Ren C, Zhang X, Li J, and Gao F. Early-life exercise induces immunometabolic epigenetic modification enhancing anti-inflammatory immunity in middle-aged male mice. Nature communications 15: 3103, 2024.
176. Liu M, and Ren T. Impact of personlized exercise prescription on muscle mass, physical function, and quality of life in postoperative pancreatic cancer patients undergoing da Vinci robotic surgery: a randomized controlled trial protocol. Trials 26: 420, 2025.
177. Gundakaram S, Sirigireddy S, McDonald A, King E, and Goparaju P. Evaluating the role of exercise in modulating immunity and immunotherapy outcomes in cancer: A systematic review. 43: 2646-2646, 2025.
178. Cheung C, Boocock E, Grande AJ, and Maddocks M. Exercise-based interventions for cancer cachexia: A systematic review of randomised and non-randomised controlled trials. Asia-Pacific journal of oncology nursing 10: 100335, 2023.
179. Allan J, Buss LA, Draper N, and Currie MJ. Exercise in People With Cancer: A Spotlight on Energy Regulation and Cachexia. Frontiers in physiology 13: 836804, 2022.

180. Peyrachon R, Lièvre A, and Rébillard A. The role of aerobic and resistance exercise for cancer cachexia management – A systematic scoping review. Asia-Pacific journal of oncology nursing 12: 100748, 2025.
181. Shaver AL, Sharma S, Nikita N, Lefler DS, Basu-Mallick A, Johnson JM, Butryn M, and Lu-Yao G. The Effects of Physical Activity on Cancer Patients Undergoing Treatment with Immune Checkpoint Inhibitors: A Scoping Review. Cancers 13: 2021.
182. Avancini A, Belluomini L, Tregnago D, Trestini I, Milella M, Lanza M, and Pilotto S. Exercise and anemia in cancer patients: could it make the difference? Expert review of hematology 14: 979-985, 2021.
183. Dolan LB, Gelmon K, Courneya KS, Mackey JR, Segal RJ, Lane K, Reid RD, and McKenzie DC. Hemoglobin and aerobic fitness changes with supervised exercise training in breast cancer patients receiving chemotherapy. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 19: 2826-2832, 2010.
184. Mohamady HM, Elsisi HF, and Aneis YM. Impact of moderate intensity aerobic exercise on chemotherapy-induced anemia in elderly women with breast cancer: A randomized controlled clinical trial. Journal of advanced research 8: 7-12, 2017.
185. Drouin JS, Young TJ, Beeler J, Byrne K, Birk TJ, Hryniuk WM, and Hryniuk LE. Random control clinical trial on the effects of aerobic exercise training on erythrocyte levels during radiation treatment for breast cancer. Cancer 107: 2490-2495, 2006.
186. Furrer R, Jauch AJ, Nageswara Rao T, Dilbaz S, Rhein P, Steurer SA, Recher M, Skoda RC, and Handschin C. Remodeling of metabolism and inflammation by exercise ameliorates tumor-associated anemia. Science advances 7: eabi4852, 2021.
187. Houston JL, Boyd KP, Gittings PM, and Cavalheri V. What are safe hemoglobin, neutrophil and platelet counts for people with hematological malignancies to participate in exercise and activities of daily living: a scoping review. Supportive Care in Cancer 33: 605, 2025.
188. Hassing Johansen S, Reinertsen KV, Wisløff T, Sæter M, Sarvari SI, Kiserud CE, Strandos N, Edvardsen E, Grydeland M, Nilsen TS, and Thorsen L. Effects of aerobic exercise on late effects and quality of life in Long-Term breast cancer survivors: a randomized controlled trial. JNCI cancer spectrum 2025.
189. Wang S. Run to boost your gut microbiome that can help fight cancer. Cell host & microbe 33: 1464-1465, 2025.
190. McTiernan A. In Motion: Experimental Evidence on Exercise and Breast Cancer in Women and Mice. Clinical cancer research : an official journal of the American Association for Cancer Research 31: 3353-3355, 2025.
191. Nates JL, Pène F, Darmon M, Mokart D, Castro P, David S, Povoa P, Russell L, Nielsen ND, Gorecki GP, Gradel KO, Azoulay E, and Bauer PR. Septic shock in the immunocompromised cancer patient: a narrative review. Critical care (London, England) 28: 285, 2024.
192. Cortiula F, Hendriks LEL, van de Worp W, Schols A, Vaes RDW, Langen RCJ, and De Ruysscher D. Physical exercise at the crossroad between muscle wasting and the immune system: implications for lung cancer cachexia. Journal of cachexia, sarcopenia and muscle 13: 55-67, 2022.
193. Chen X, Li Z, and Zhang J. A novel approach to cancer rehabilitation: assessing the influence of exercise intervention on postoperative recovery and survival rates. International journal of surgery (London, England) 111: 3373-3385, 2025.
194. Wen H, Zhang P, Zhao J, Liu Y, Wan L, Li H, Yi J, and Li X. Metabolic alterations driven by LDHA in CD8+T cells promote immune evasion and therapy resistance in NSCLC. Scientific reports 15: 24440, 2025.
195. Miao R, Liu C, Wang Y, Li H, Han C, Wang C, Tian Z, and Liu J. Exercise-induced lactate suppresses ccRCC via CNDP2-mediated depletion of intracellular amino acids. Cell Death Discovery 11: 356, 2025.
196. Navarro-Ledesma S, Hamed-Hamed D, González-Muñoz A, and Pruimboom L. Physical Activity, Insulin Resistance and Cancer: A Systematic Review. Cancers 16: 2024.
197. Rangel M, Lopes KG, Qin X, and Borges JP. Exercise-induced adaptations in the kynurenine pathway: implications for health and disease management. Frontiers in sports and active living 7: 1535152, 2025.
198. Gustafson MP, Wheatley-Guy CM, Rosenthal AC, Gastineau DA, Katsanis E, Johnson BD, and Simpson RJ. Exercise and the immune system: taking steps to improve responses to cancer immunotherapy. Journal for immunotherapy of cancer 9: 2021.
199. Koelwyn GJ, Wennerberg E, Demaria S, and Jones LW. Exercise in Regulation of Inflammation-Immune Axis Function in Cancer Initiation and Progression. Oncology (Williston Park, NY) 29: 908-920, 922, 2015.
200. Orange ST, Leslie J, Ross M, Mann DA, and Wackerhage H. The exercise IL-6 enigma in cancer. Trends in endocrinology and metabolism: TEM 34: 749-763, 2023.

Immunometabolic profiling of T cells in response to prolonged moderate intensity cycling in humans

Fendi Pradana [1,2], Jonathan P Barlow [1,3], Jack Shayler [1], Sarah K Dimeloe [4,5], Nancy Gudgeon [4,5], Tim Podlogar [1], Gareth A Wallis [1], Alex J Wadley [1*]

[1] School of Sport, Exercise, and Rehabilitation Sciences, University of Birmingham, UK
[2] Nutrition Study Program, Universitas Tadulako, Indonesia
[3] Cellular Health & Metabolism Facility, University of Birmingham, UK
[4] Institute of Immunology and Immunotherapy, College of Medical and Dental Sciences, IBR, University of Birmingham, UK
[5] Institute of Metabolism and Systems Research, University of Birmingham, UK

ABSTRACT

Background: Emerging data indicates that enrichment of peripheral blood with T lymphocytes during exercise and their associated changes in function are underpinned by modulation of cellular bioenergetics. However, there is a dearth of literature examining these responses using metabolic thresholds to prescribe exercise intensity or providing single cell resolution on immunometabolic outcome measures. Objectives: The current study was designed to examine the metabolic phenotypes and real-time bioenergetic responses to activation of enriched naïve helper ($CD4^+$) and cytotoxic ($CD8^+$) T cells and peripheral blood mononuclear cells (PBMCs) in response to prolonged cycling. Methods: Ten aerobically trained males and females (mean ± SD: age 21 ± 1 years; maximal oxygen consumption: 53.9 ± 9.8 ml · kg^{-1} · min^{-1}) undertook a 2-hour bout of continuous cycling at a power output eliciting 95% lactate threshold-1. Blood samples were collected at rest, immediately (post), and 2 hours after cycling cessation (recovery). Using injection sequences of cell respiration modulators and a CD3/CD28 activator, bioenergetic profiles of PBMCs and enriched naïve $CD4^+$ and $CD8^+$ T cells were determined using extracellular flux analysis. Mitochondrial membrane potential ($\Delta\Psi m$) was examined using flow cytometry. Results: Despite cycling evoking significant fluctuations in peripheral blood T cell numbers, there were no changes in absolute or relative measures of mitochondrial respiration, glycolytic flux and ATP synthesis rate post and recovery vs rest. Contribution of mitochondrial respiration to ATP production was greater than glycolysis in naïve T cells across all timepoints, but not PBMCs in recovery. This was despite absolute and relative changes in $\Delta\Psi m$ of memory T cells being greater in recovery vs. rest. Bioenergetic responses to ex vivo T cell activation were not different between cell types or timepoints. Conclusion: These data indicate that the metabolic phenotypes of naïve T cells and PBMCs were largely unaltered within 2 hours of prolonged moderate intensity cycling.

**Corresponding author:*
Dr Alex J Wadley
School of Sport, Exercise & Rehabilitation Sciences
University of Birmingham
Edgbaston
Birmingham, UK
B15 2TT
Email: a.j.wadley@bham.ac.uk
Phone: +44 (0)121 414 8011

INTRODUCTION

Single bouts of exercise promote the preferential mobilisation of lymphocytes primed for migration to central and peripheral tissues. These include skeletal muscle (16) and work in animals indicates that lymphoid sites of foreign antigen encounter, such as the lung and Peyer's patches accumulate T cells after exercise cessation (37). Trafficking of lymphocytes in this manner is believed to facilitate remodelling of these tissues; however, the underpinning mechanisms are unclear. In 1958, it was first reported using respirometry that moderate intensity exercise increased peripheral blood mononuclear cell (PBMC) oxygen consumption in humans (5). Recent advances in real-time extracellular flux analysis (EFA) have permitted more comprehensive profiling of substrate metabolism from intact immune cells by measuring oxygen flow in response to various mitochondrial agents. In PBMCs isolated immediately after vs. before exercise, it has been reported that swimming exercise to exhaustion increased basal respiration (72) and low-intensity cycling (~35% $\dot{V}O_{2peak}$)increased basal and fatty acid-dependent respiration (41). Other studies have reported no changes in PBMC respiratory states immediately after steady state (75) and exhaustive exercise (72), notably when normalised to the number of PBMCs within peripheral blood. PBMCs are composed of diverse sub-populations of lymphocytes and monocytes with unique functions and distinct metabolic profiles (8, 12, 20, 46, 66). Moreover, bouts of exercise evoke an immediate increase in peripheral blood lymphocyte concentrations, followed by a decrease in recovery (≈1-3 hours) as differentiated lymphocytes with a cytotoxic phenotype leave the bloodstream. This presents a challenge when sampling cells for evaluation of their metabolism and conclusions have largely been drawn from mixed cell populations, i.e., PBMCs. One study by Lu et al, 2022 demonstrated that maximal and reserve oxygen consumption rate (OCR) were increased in immunomagnetically enriched natural killer (NK) cells isolated from the PBMC fraction after vs. before exhaustive cycling. This study also reported that after 6 weeks of regular exercise training, maximal and reserve OCR were increased in NK cells at rest (42). Other studies have similarly documented a relationship between aerobic fitness and basal and maximal PBMC respiration (2, 32) and mitochondrial mass was higher in naïve cytotoxic ($CD8^+$) T cells isolated from active vs. inactive individuals (1). Immune cell metabolism is evidently remodelled with exercise training; however, due to marked compositional shifts in PBMCs, limited work has examined cell-by-cell bioenergetic changes in response to single bouts of exercise, notably in T cells.

Helper ($CD4^+$) T cells serve broadly to orchestrate immune response via cytokine signalling (e.g., interleukin (IL)-2, interferon-gamma and tumour necrosis factor-alpha), whereas $CD8^+$ T cells eliminate cancerous or virus infected cells through the release of cytotoxic molecules. It is now established that cellular metabolism is modulated to meet the bioenergetic and biosynthetic demands needed to govern these T cell processes (81). The metabolic pathways used to generate ATP in T cell subsets are dependent on the degree of antigen experience (56). Naïve T cells are antigen inexperienced cells generated in the thymus that mostly rely on mitochondrial respiration to fulfil their primary function of recirculating through lymphoid tissues to survey for antigens (47). Upon T cell receptor (TCR) engagement and co-stimulatory CD28 ligation, a pronounced glycolytic shift facilitates T cell effector functions and subsequent differentiation into memory T cells (24, 57, 80). Antigen encounters therefore result in central memory (CM), effector memory (EM) and terminally differentiated effector memory (TEMRA) T cells exhibiting increasingly greater reliance on glycolysis than naive T cells for basal respiration (TEMRA > EM > CM > naïve). Enrichment of peripheral blood with antigen experienced T cells with higher basal respiration may therefore influence the metabolic activity of the PBMC fraction during exercise and make changes within individual T cell subsets difficult to discern (72). Perturbations in T cell energetics are permissible given the multiple immunological stressors associated with exercise at the cellular, systemic, and tissue level. A recent study employing single cell RNA sequencing reported that genes associated with metabolic regulation were enriched in $CD4^+$ and $CD8^+$ T cells after maximal exercise, most notably in EM (3). These changes were aligned with upregulation of genes associated with cell migration, antigen binding, and cytokine production. Other data indicate that exercise-mobilised T cells are primed to uptake energy substrates such as glucose (75), and this may facilitate modulation of their metabolic activity to fulfil these effector functions.

Most studies examining immunometabolic responses to exercise have evaluated changes in PBMCs immediately after bouts ≤1 hour. A recent scoping review specifically highlighted that there is a dearth of literature examining immunometabolic responses in individual T cell subsets after prolonged exercise into recovery (63). By using prior cell sorting, EFA can provide this single cell resolution (38, 58, 64). Furthermore, contemporary flow cytometry permits interrogation of cellular metabolism by coupling metabolic indicators of mitochondrial membrane potential ($\Delta\Psi m$) to T cell immunophenotyping, providing cell-by-cell measurements (25).

This study was designed to examine the metabolic sensitivity of T cell subsets to prolonged moderate intensity cycling compared to the PBMC fraction. Naïve $CD4^+$ and $CD8^+$ T cells were a specific focus of EFA given that these cells are mobilised into peripheral circulation during exercise, abundant within the PBMC fraction and metabolically adaptable to exercise training (1), thus providing a robust single cell model to examine immunometabolic responses to exercise. Previous studies in this area have largely prescribed exercise intensity based on a fixed proportion of maximal oxygen consumption ($\dot{V}O_{2max}$) (32, 42); however, this doesn't account for inter-individual variability in substrate uptake and oxidation during exercise, which might govern subsequent immunometabolic responses. Prescribing intensity at the upper limit of moderate exercise intensity domain, demarcated by lactate threshold-1 (LT1 - exercise intensity at which there is a measurable increase in blood lactate concentration) would facilitate comparable relative metabolic stress between participants (30, 31, 40). Accordingly, the aims of this study were to 1) evaluate the metabolic phenotypes of immunomagnetically enriched naïve $CD4^+$ and $CD8^+$ T cells and PBMCs using extracellular flux analysis; 2) quantify $\Delta\Psi m$ of these T cell subsets using flow cytometry; 3) examine real-time metabolic, morphological and cytokine responses to *ex vivo* T cell activation and 4) draw as-

sociations of these outcomes with circulating T cell substrates (glucose, glutamine, and triglycerides) in response to 2 hours of cycling at 95% LT1.

MATERIALS AND METHODS

Participants

Ten participants (5 males and 5 females) gave written informed consent to take part in this study (Table 2). Participants underwent screening prior to enrolment to include individuals who were physically active as defined by the General Practice Physical Activity Questionnaire (GPPAQ) (21), and exceeded an aerobic fitness threshold for males (> 50 ml · kg^{-1} · min^{-1}) and females (> 35 ml · kg^{-1} · min^{-1}) (77). All experimental visits for female participants were conducted at the same phase of menstrual cycle to prevent the influence of estrogen on immunometabolic markers (52). Participants were excluded if they were smokers, currently taking medication/s, eating a ketogenic diet, had donated blood in the last 3 months, or had a history of cardiovascular, metabolic, neurological, or inflammatory disease.

Study Design

The study was a randomised crossover design, comprising four morning visits at the School of Sport, Exercise and Rehabilitation Sciences at the University of Birmingham, conforming to the Declaration of Helsinki, except for prior registration on a publicly accessible database (85). After an initial screening visit to determine LT and $\dot{V}O_{2max}$, participants undertook three randomised visits, consisting of two identical cycling trials (CT-1 and CT-2) and one rest trial (REST), each separated by 7 days (Figure 1). Our preliminary data indicated differences in real-time glycolytic responses to T cell activation based on blood sample processing (0-4 hours after collection) and temperature (room temperature or 37°C) of metabolic assays (data not shown). Given that T cell metabolism was to be examined over a 4-hour period, the study protocol was designed to equalise time spent preparing T cell metabolic assays and eliminate the effect of blood 'sitting time'. Therefore, primary blood samples were collected on different trials at rest or 'Pre-Ex' (REST), immediately after or 'Post-Ex' (CT-1), and 2 hours after or 'Recovery' (CT-2) cycling and processed immediately at room temperature. CT-1 and CT-2 were differentiated only by the timing of these blood sample withdrawals after cycling cessation, and conducted under identical conditions, with the aim of eliciting comparable physiological and immunological responses. These were confirmed by monitoring gas exchange data and drawing secondary blood samples at every timepoint throughout CT-1 and CT-2 respectively (details below). Between all trials, lifestyle factors that might influence immunity were subjectively monitored, including illness symptoms, anxiety, and sleep quality.

All visits started at the same time of day for each participant (range: 6:30–8.00 AM) and were conducted under stable climatic conditions (temperature (°C): 21.0 ± 0.6, humidity (%): 39.1 ± 10.0 and barometric pressure (hPa): 1002.1 ± 12.7). Participants were asked to refrain from vigorous exercise, and the consump-

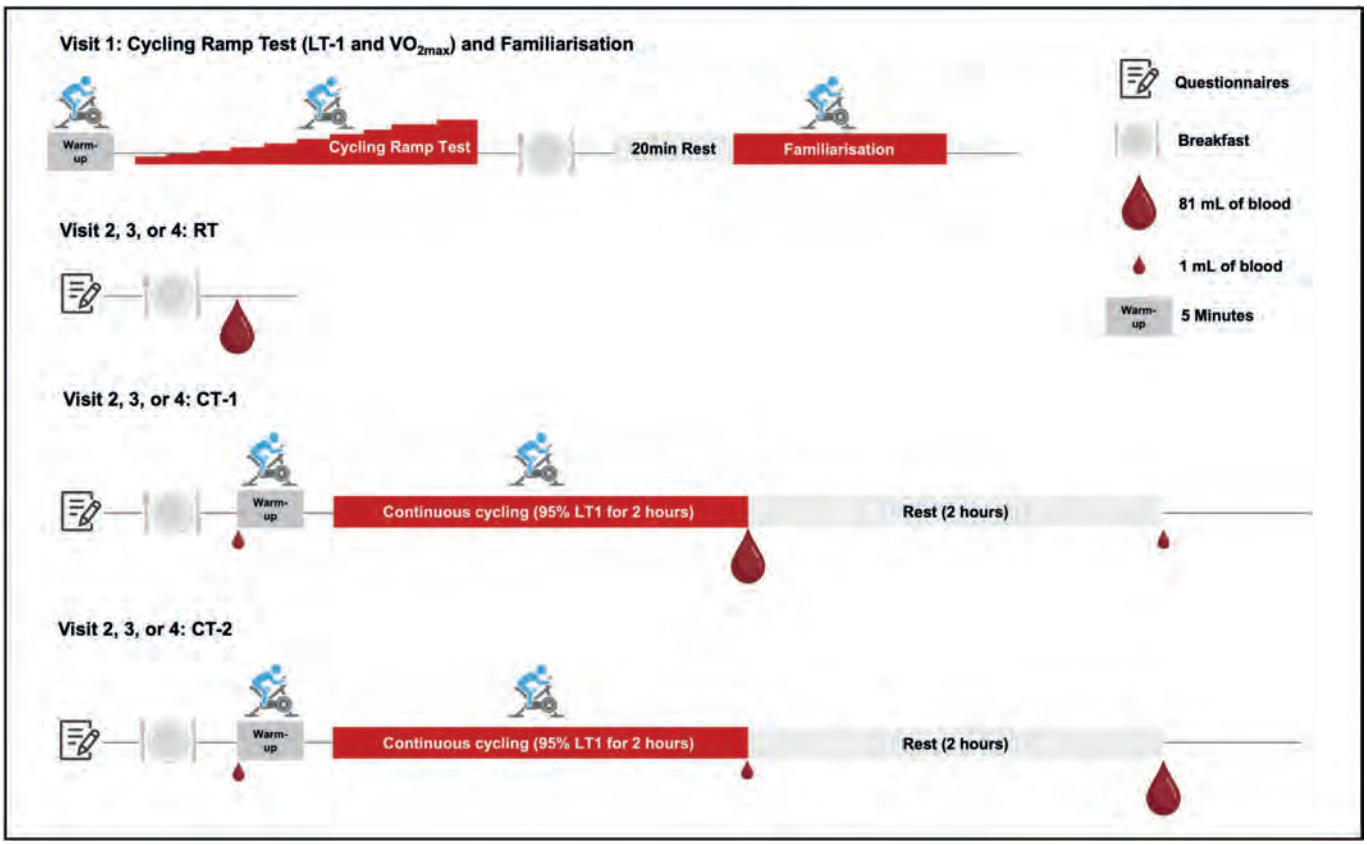


Figure 1. Study schematic illustrating a time axis for each of the 4 laboratory visits. On visit 1, a cycling ramp test to exhaustion was used to determine lactate threshold-1 (LT1) and maximal oxygen consumption (VO_{2MAX}). Participants then undertook 3 randomised trials, including Rest (RT) and cycling trials 1 and 2 (CT-1 and CT-2). Blood sampling is indicated with small droplets (1 mL) for samples collected for determination of complete blood cell count and large droplets (77 mL) for all other outcomes. Key indicates when breakfast, questionnaires, warm-up and rest periods took place. Created with BioRender.com.

tion of caffeine and alcohol for 48 hours prior to attending each visit. Furthermore, to standardise nutrition, participants were asked to record their food intake for 24 hours before the first visit and to replicate this diet for all subsequent visits, as well undertaking an overnight fast from 10pm, consuming only water (ad libitum) during this period. Participants were provided with a breakfast of oats mixed with semi-skimmed milk (normalised for carbohydrate content: 1 g · kg^{-1} body mass) (6, 76) to ensure the energy and macronutrient intake across trials and between participants was standardised.

Screening Visit (Visit 1)

Participants attended the laboratory for screening and determination of LT and $\dot{V}O_{2max}$. Body mass (Ohaus CD31, New Jersey, USA), height (Seca Alpha, Hamburg, Germany) and resting blood pressure (Thuasne BP 3W1-A, Taipei, Taiwan) were recorded. An exercise tolerance test was then conducted on an electromagnetically braked cycle ergometer (Excalibur, Lode, Netherlands). After a warm-up for 5 minutes at a rating of perceived exertion (RPE) of 8–10 using the Borg scale (61), participants commenced cycling at 70 watts and then 30-watt increments were added every 4 minutes until volitional exhaustion (RPE = 20). A breath-by-breath system (Vyntus, Vyaire Medical, IL, US) was used for continuous measurement of oxygen uptake, and fingertip blood lactate measurements were made at the end of every 4-minute stage (Lactate Pro 2, Arkray, Kyoto, Japan). Participants were asked to maintain a pedal cadence > 60 and encouragement was given by the research team. $\dot{V}O_{2max}$ was calculated using gas exchange data from the final stage and expressed relative to body weight (ml · kg^{-1} · min^{-1}) (28). Participants were provided with breakfast and during this time, LT software (36) was used to determine the power output that elicited a 0.5 mmol · L^{-1} increase in lactate above baseline value, thus defining LT1 (30, 31). A 15-minute familiarisation was then conducted at a power output eliciting 95% of LT1 and a final blood lactate measurement made to confirm the correct intensity for subsequent trials.

Experimental Trials (Visits 2-4)

In the morning of each trial (REST, CT-1, and CT-2), participants were asked to complete questionnaires evaluating illness symptoms (48) state and trait anxiety (71) and sleep efficiency (percentage of time asleep relative to the amount of time spent in bed) (11) during a 30-minute period of rest (Pre-Ex). During this time, blood pressure and body mass were also measured. A standardised breakfast was provided as described above and then a catheter (Becton, Dickson & Company, Oxford, UK) inserted into the antecubital vein of the forearm to obtain a resting blood sample 15 minutes after feeding. Each exercise trial commenced with a 5-minute warm-up at a RPE of 8–10, followed by 2-hours of continuous-cycling at a power output eliciting 95% LT1 (CT-1 or CT-2). Every 15 minutes during CT-1 and CT-2, exercise intensity was confirmed by measuring $\dot{V}O_2$ uptake for 3 minutes and heart rate (H10, Polar Electro, Finland), RPE and the affective response (26) recorded. Energy expenditure, carbohydrate and fat oxidation were calculated based on gas exchange data (33). Following completion of CT-1 and CT-2, participants remained seated in the laboratory for a 2-hour recovery period.

Blood Sampling

A total of seven blood samples were taken across the 3 trials, including a single blood draw during REST (Pre-Ex) and 3 blood draws during CT-1 and CT-2 (Pre-Ex, Post-Ex, and Recovery). The catheter was kept patent through regular flushes with saline (0.9% NaCl, Becton, Dickson & Company, Oxford, UK). The volumes of blood drawn at each timepoint varied depending on the trial (Figure 1). During REST (Pre-Ex), immediately after CT-1 (Post-Ex) and 2 hours post-exercise in CT-2 (Recovery), 70 mL of blood was collected into sodium heparin vacutainers (Becton, Dickson & Company, Oxford, UK) for isolation of peripheral blood mononuclear cells (PBMCs). Additionally, 6 mL of blood was collected into K_2EDTA vacutainers (Becton, Dickson & Company, Oxford, UK) and 4 mL into clotting vacutainers (Becton, Dickson & Company, Oxford, UK) for isolation of plasma and serum at these 3 timepoints, respectively. Across all seven timepoints, 1 mL of blood was collected into K_2EDTA vacutainers (Greiner Bio-One, Frickenhausen, Germany) to obtain a complete blood cell count using an automated haematology analyser (Yumizen H500, Horiba, Kyoto, Japan). These data were obtained to ensure comparable leukocyte counts at rest (REST vs. CT-1 vs. CT-2) and in response to prolonged cycling (CT-1 vs. CT-2).

Blood Processing and Immunomagnetic Separation of Naïve T Cells

PBMCs were isolated by density gradient centrifugation and then naïve $CD4^+$ and $CD8^+$ T cells enriched using magnetic activated cell sorting (MACS) microbead isolation kits (Cat #130-045-101 and Cat #130-093-244, Miltenyi Biotec, Bergisch Gladbach, Germany) (82). Purity of each cell fraction was confirmed using flow cytometry. All methods and the gating strategy for naïve, CM, EM and TEMRA T cells are presented in Supplemental Figure 1. Whole blood in clotting tubes was left for 15 minutes at room temperature and then centrifuged alongside K_2EDTA blood collection tubes for 10 minutes at 1,525 x g at 4 °C for isolation of serum and plasma respectively. Both serum and plasma were then aliquoted and stored at -80 °C until subsequent analysis.

Immunophenotyping and Assessment of Mitochondrial Membrane Potential

Assay Conditions

Fluorescently conjugated antibodies and the mitochondrial indicator (MI) MitoSpy Orange chloromethyl-tetramethylrosamine (MSO, Cat# 424804, Biolegend) were combined to quantify the $\Delta\Psi m$ of naïve, CM, EM and TEMRA $CD4^+$ and $CD8^+$ T cell subsets using nine-colour flow cytometry (Beckman Coulter, California, USA) (25, 54). Peripheral blood T cell concentrations (cells/µL) were also determined and adjusted for changes in blood volume (44). MSO is a cationic compound that passively diffuses across the plasma membrane and accumulates in mitochondria based on its electrochemical gradient, thus serving as a surrogate for mitochondrial activity. A separate sample was stained under identical conditions, but with BAM-15 to uncouple mitochondria and permit measurement of baseline MSO mean fluorescence intensity (MFI). MSO was monitored by the λ610 nm laser and λ585/42nm detector. MitoView Green (Cat # 70054, MVG, Cambridge Biosciences) was monitored on the λ488 nm laser and λ525/40nm detector to quantify mitochondrial mass. Full details of antibody and MI staining and flow cytometry gating are described in supplementary materials.

Calculations (Cell concentrations and ΔΨm)

Peripheral blood cell concentrations were calculated by combining automated haematology analysis (lymphocyte concentration)

with individual T cell subset frequencies determined by flow cytometry. All concentrations were adjusted for changes in blood volume using the formula proposed by Matomäki et al, 2000 (44) and expressed as cells per µL. Within each cell subset, ΔΨm was expressed as a ratio of the raw MFI of MSO to the BAM-15 treated control. Both absolute and relative changes (vs. Pre-Ex) in ΔΨm were then calculated. Mitochondrial mass was monitored across timepoints by quantifying the MFI of MVG within each T cell subset.

Real-time metabolic profiling

Experimental assays were carried out using the Seahorse XFe96 extracellular flux analyser and pre-designed using Seahorse analytics software 1.0.0-570 (Agilent Technologies, USA). A metabolic profiling assay (Figure 3A–B) was used to examine the bioenergetic profile of PBMCs, enriched naïve $CD4^+$ and $CD8^+$ T cells (via OCR and ECAR measurement) using an injection sequence of different cell respiration modulators (Oligomycin, BAM 15, Rotenone and Antimycin A) (45). A T cell activation assay (Figure 5C–E) was coupled to this by injecting anti-human CD3/CD28 soluble antibody complexes to evaluate real time bioenergetic responses, measured via changes in PER and OCR (35, 47). The assay preparation and injection strategies are outlined separately below for simplicity.

Mito Stress Assay

Preparation

A Seahorse XFe96 sensor cartridge was hydrated in 200 µL/well of XF Calibrant in a non-CO_2 incubator overnight at 37°C. The day after, Seahorse XFe96 extracellular flux analyser was calibrated for minimum 5 hours prior to starting the assay. PBMCs, enriched naïve $CD4^+$ and $CD8^+$ T cells (2 x 10^5 cells/well) were suspended in 50 µL of pre-warmed Seahorse XF RPMI assay medium (supplemented with 10 mM glucose, 1 mM pyruvate, and 2 mM glutamine, pH = 7.4, Agilent Technologies, USA) and seeded onto a Seahorse XFe96 cell culture microplate (Agilent Technologies, USA). A total of 4 technical replicates were used for each assay. Each well was pre-coated with sterile Cultrex Poly-D-lysine (Bio-techne, Minneapolis, USA). The plate was centrifuged at 300 x g for 5 minutes at room temperature with the brake off and the plate rested for 1 hour in a non-CO_2 incubator at 37°C. Assay medium (130 µL) was added 15 minutes prior to starting experiments.

Assay Injection Strategy

Injected reagents (20 µL/well) were prepared including 2 µg · mL^{-1} Oligomycin (Sigma-Aldrich, Merck, UK) in port A, 3 µM BAM 15 (TOCRIS, Minneapolis, USA) in port B and a mixture of 2 µM Rotenone + 2 µM Antimycin A (Sigma-Aldrich, Merck, UK) in port C. The experimental plate was then inserted into the analyser, and an induced real-time ATP rate (pmol · min^{-1}) assay was performed. Following the pre-design experimental assay, OCR (pmol · min^{-1}) and ECAR (mpH · min^{-1}) were measured 14 minutes after the assay begun reflecting the baseline measurement (3 cycles) and following each of 3 consecutive injections over a 40-measurement period (Figure 3B). Injections of Oligomycin (port A, 3 cycles) after 15–28 minutes, BAM 15 (port C, 3 cycles) after 29–41 minutes and a mixture of Rotenone + Antimycin A (port C, 3 cycles) after 42–54 minutes were implemented to provide a detailed metabolic profile including mitochondrial respiration, glycolytic and ATP synthesis rate for each sample.

Calculations

Data are resented in absolute values per 2 x 10^5 cells as OCR (pmol O_2 · min^{-1}), Glycolytic PER (pmol H^+ · min^{-1}) and ATP synthesis rate (pmol ATP · min^{-1}). Calculations were then performed to define respiratory parameters, which are provided in Table 1 and visually depicted in Figure 3A. Absolute values for naïve $CD4^+$ and $CD8^+$ T cells were coupled to T cell frequencies determined by flow cytometry to calculate the contribution of naïve $CD4^+$ and $CD8^+$ T cells to each metabolic outcome within the PBMC fraction. In addition, relative contributions of OCR (% of total and % of maximum) and ATP synthesis rate (% of total) were determined separately for each cell fraction.

Table 1. Calculation of respiratory parameters measured by Seahorse extracellular flux analyser

Parameter	Calculation
Basal mitochondrial respiration	OCR without any injections minus OCR after addition of rotenone and antimycin A.
Proton leak	OCR after oligomycin injection minus OCR after addition of rotenone plus antimycin A.
Maximal mitochondrial respiration	OCR after injection of BAM15 minus OCR after addition of rotenone plus antimycin A
Spare respiratory capacity	Maximal mitochondrial respiration minus basal mitochondrial respiration
ATP-linked respiration	Basal mitochondrial respiration minus proton leak
Non-mitochondrial respiration	OCR after the injection of rotenone plus antimycin a
Mitochondrial PER	Basal mitochondrial respiration (OCR) multiplied by 0.61 (CO_2 contribution factor)
Glycolytic PER	Total PER minus mitochondrial PER
Mitochondrial ATP synthesis rate	ATP-linked respiration (corrected for a 10% overestimation of proton leak due to oligomycin-induced hyperpolarisation of mitochondrial inner membrane) multiplied by 5.45 (P/O_2 ratio for glucose) plus basal mitochondrial respiration multiplied by 0.242 (P/O_2 ratio for TCA flux). We have accounted for mitochondrial ATP driven by reducing equivalents generated during both glycolysis and the oxidation reactions of pyruvate dehydrogenase plus the tricarboxylic acid cycle, and substrate-level phosphorylation at succinyl-CoA synthetase during activity of the tricarboxylic acid cycle.
Glycolytic ATP synthesis rate	Glycolytic PER (pmol H^+/min) using 1:1 stoichiometry for H^+ and lactate. We did not include pyruvate oxidation to bicarbonate in glycolytic ATP calculations.
ATP synthesis rate	Glycolytic ATP synthesis rate + mitochondrial ATP synthesis rate
Abbreviations: OCR, oxygen consumption rate; ATP, adenosine triphosphate; PER, proton efflux rate; P/O ratio, phosphorylated per atom of oxygen.	

T cell Activation Assay

Assay Injection Strategy

A T cell activation assay was used to examine real-time metabolic responses to activation and followed identical preparation procedures to the Mito stress assay. PER (pmol · min^{-1}) and OCR (pmol O_2. min^{-1}) were measured 14 minutes after the assay baseline measurement (3 cycles) and following each of 2 consecutive injections over a 100-minute period (Figure 5C–E). A human Im-

munoCult CD3/CD28 activator (Catalog # 10991, STEMCELL Technologies, Cambridge, UK) or assay media were injected at 20 µL/well (port A, 10 cycles) after 15–79 minutes and then the glucose analog 2-Deoxy-D-glucose (2-DG, Thermo Scientific, UK) injected after 80–120 minutes (port B, 4 cycles). The injection of 2-DG caused a rapid inhibition of glycolysis and subsequent decrease in PER, thus providing confirmation that prior changes in PER were primarily due to glycolysis (70).

Calculations

Data were presented in absolute values per 2 x 10^5 cells as Glycolytic PER (pmol $H^+ \cdot min^{-1}$), ATP-linked respiration based on OCR (pmol $O_2 . min^{-1}$), and ATP synthesis rate (pmol ATP $\cdot min^{-1}$). Data were calculated following the formula provided in Table 1 and visually depicted in Figure 5C–E).

Ex Vivo T-cell Stimulation

Under sterile conditions, PBMCs and enriched naïve $CD4^+$ and $CD8^+$ cells (2 x 10^5 cells/well) were suspended in 180 µL of pre-warmed ImmunoCult-XF T-cell expansion medium (STEMCELL Technology, UK) and seeded onto a non-treated 96-well round bottomed microplate (Fisher Scientific, UK). Into each well, 20 µL of ImmunoCult human CD3/CD28 T-cell activator (Catalog # 10991, STEMCELL Technologies, Cambridge, UK) or 20 µL of expansion medium (control well) was gently mixed with the cells and incubated for 12 hours at 37 °C (5% CO_2). All cell suspensions were centrifuged at 300 x g for 5 minutes at room temperature to harvest cells for measurement of post-activation diameter (µM) using a dual fluorescence cell counter (Nexcelom Bioscience, Massachusetts, USA), and quantify interleukin 2 (IL-2) in the supernatant from activated naïve T cells using high sensitivity ELISA kits (Bio-techne, Minneapolis, USA, Cat# HS200).

Metabolic Substrates

The concentrations of glucose and glutamine in plasma, and triglyceride in serum were quantified at all timepoints using bioluminescent rapid assay kits (Promega, Madison, USA). Lactate was measured at Rest and throughout CT-1 and CT-2, but not Recovery (Lactate Pro 2, Arkray, Kyoto, Japan).

Statistical Analysis

GraphPad Prism 10.2.2 analysis software (San Diego, CA) was used to perform statistical analysis and graph creation. Data were assessed for normal distribution using the Shapiro-Wilk test. Normally distributed variables were analysed across exercise trials (REST, CT-1, and CT-2), timepoints (Pre-Ex, Post-Ex, and Recovery) and cell types (PBMCs, naïve $CD4^+$ and $CD8^+$ T cells) by mixed-effects two-way analysis of variance (Two-way ANOVA). Post hoc analyses of any interaction effects (e.g., Trial x Time or Time x Cell Type) were performed by a test of multiple comparisons, with either Tukey test, depending on variable normality. One-way analysis of variance (One-way ANOVA) was used to analyse peripheral blood immune cell concentrations, immunometabolic outcomes and metabolic substrates between timepoints (Pre-Ex, Post-Ex, and Recovery). In addition, t-tests were used to analyse the differences in physiological data between CT-1 and CT-2. Data that were not normally distributed were analysed using Wilcoxon or Kruskal-Wallis's test. Statistical significance was accepted at the $p < 0.05$ level. All values are presented as means ± standard deviation (SD). To assist with interpretation of changes in immunometabolic outcomes, 95% confidence intervals (CI) and effect sizes (Cohen's d) were computed and presented in Supplementary Materials. Effect sizes of 0.2, 0.5 and 0.8 were considered small, moderate and large respectively (13, 43).

RESULTS

Participant Characteristics, Sleep Efficiency and Anxiety

Mean participant characteristics including anthropometrics, cardiorespiratory fitness, maximal and LT1 power output are reported in Table 2. A repeated measures ANOVA showed no significant differences in sleep efficiency ($F_{1,11} = 1.87$, $p = 0.20$), state anxiety ($F_{2,15} = 0.69$, $p = 0.49$) or trait anxiety ($F_{2,17} = 0.23$, $p = 0.79$) (Table 3). Sleep efficiency and anxiety were therefore not included as covariates in subsequent statistical analyses of primary and secondary variables.

Physiological Responses to Exercise and Workload Measurements

Physiological data, estimates of energy expenditure and subjective perceptions during CT-1 and CT-2 are presented in Table 4. A paired T-test revealed no significant differences in average HR ($t(9) = 0.83$, $p = 0.43$), absolute $\dot{V}O_2$ uptake ($t(9) = 0.84$, $p = 0.42$), relative $\dot{V}O_2$ uptake ($t(9) = 0.84$, $p = 0.42$), total energy expenditure ($t(9) = 0.38$, $p = 0.71$), carbohydrate oxidation ($t(8) = 0.22$, $p = 0.83$), fat oxidation ($t(8) = 0.30$, $p = 0.77$), affective response ($t(9) = 1.77$, $p = 0.11$) or RPE ($t(9) = 0.56$, $p = 0.59$) between CT-1 and CT-2, demonstrating consistent physiological

Table 2. Participant characteristics

Variable	Male (n = 5)	Female (n = 5)
Age (years)	20.60 ± 1.14	20.80 ± 1.10
Height (cm)	185.70 ± 5.94	164.80 ± 4.16
Body mass (kg)	69.81 ± 9.03	61.89 ± 5.07
BMI ($kg \cdot m^2$)	20.17 ± 1.47	22.79 ± 1.70
$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	60.26 ± 2.74	47.63 ± 10.40
Maximum Power output (W)	299.10 ± 43.74	225.78 ± 33.61
Power output at LT-1 (W)	156.42 ± 31.54	142.40 ± 23.29

For consistency of all tables, SDs and significance lists have 1-line spacing form abbreviation. Example: Data displayed as mean ± SD.

Abbreviations: BMI, body mass index; $\dot{V}O_{2max}$, maximum rate of oxygen uptake; LT, lactate threshold.

Table 3. Sleep efficiency and state and trait anxiety score prior to each experimental trial

	Experimental Trial			
Variable	**RT**	**CT-1**	**CT-2**	**p-Value**
Sleep Efficiency (%)	83.80 ± 11.74	83.83 ± 8.06	88.60 ± 4.48	> 0.05
Anxiety State ($S_{anxiety}$)	44.40 ± 2.07	45.80 ± 4.87	45.40 ± 3.41	> 0.05
Anxiety Trait ($T_{anxiety}$)	45.00 ± 5.50	45.10 ± 5.26	44.20 ± 3.97	> 0.05

Data displayed as mean ± SD. $p > 0.05$ indicates no significant differences between trials.

Abbreviations: RT, rest trial; CT-1, cycling trial 1; CT-2, cycling trial 2.

and metabolic responses between trials.

Table 4. Mean Physiological responses during identical cycling trials

Experimental Trial			
Variable	**CT-1**	**CT-2**	**p-Value**
HR (bpm)	137 ± 16	135 ± 16	> 0.05
RPE	11.05 ± 1.92	10.84 ± 1.54	> 0.05
Affective Response	2.10 ± 1.13	2.50 ± 0.64	> 0.05
Average $\dot{V}O_2$ Uptake ($mL \cdot min^{-1}$)	2311 ± 337	2268 ± 333	> 0.05
Total Energy Expenditure (kcal)	1363 ± 207	1351 ± 198	> 0.05
Relative $\dot{V}O_2$ Uptake ($\%\dot{V}O_{2max}$)	67 ± 11	66 ± 11	> 0.05
Respiratory Exchange Ratio	0.89 ± 0.03	0.90 ± 0.02	> 0.05
Carbohydrate Oxidation (g/min)	1.86 ± 0.36	1.86 ± 0.38	> 0.05
Fat Oxidation (g/min)	0.41 ± 0.16	0.38 ± 0.12	> 0.05

Data displayed as mean ± SD. p > 0.05 indicates no significant differences between trials.

Abbreviations: CT-1, cycling trial 1; CT-2, cycling trial 2; HR, heart rate; RPE, rating of perceived exertion; $\dot{V}O_2$, rate of oxygen uptake.

Effects of CT-1 and CT-2 on Total White Blood Cell Concentrations

The average blood volume adjusted total white blood cell concentrations throughout CT-1 and CT-2 are displayed in Table 5. Total WBC ($p < 0.001$), neutrophil ($p < 0.001$) and monocyte ($p < 0.01$) concentrations significantly increased Post-Ex and remained elevated at Recovery. There was no significant increase in lymphocyte concentration Post-Ex ($p = 0.33$), and concentrations were similar between Pre-Ex and Recovery. There were no significant differences in any subset between trials or timepoint ($p > 0.05$), demonstrating consistent immunological responses between CT-1 and CT-2.

Effects of Cycling on T Cell Concentrations

To further examine the composition of the lymphocyte population, specifically T cell memory subsets, flow cytometry was subsequently employed. Blood volume adjusted concentrations of $CD4^+$ and $CD8^+$ T cells and their sub-populations (N, CM, EM, and TEMRA) are reported in Table 5 across REST, CT-1 and CT-2 to represent Pre-Ex, Post-Ex and Recovery respectively. There were increases in total $CD3^+$ ($p = 0.03$) and $CD8^+$ ($p = 0.008$), but not $CD4^+$ ($p = 0.07$) T cell concentrations. Within the $CD8^+$ population, these changes were driven by naïve ($p < 0.05$), EM $CD8^+$ ($p = 0.03$), and TEMRA $CD8^+$ ($p = 0.02$), but not CM T cells ($p = 0.33$). The concentrations of all $CD4^+$ ($p = 0.02$) and $CD8^+$ ($p < 0.04$) T cell subsets, except $CD4^+$ EM, significantly decreased at Recovery relative to Post-Ex, but these were not different to Pre-Ex ($p > 0.05$).

Table 5. Differences in Peripheral Blood Immune Cell Concentrations (cells/μL)

Experimental Trial				
Immune cell subset	**Pre-Ex**	**Post-Ex**	**Recovery**	**p-Value**
WBCs	5700 ± 1080[1,2]	9330 ± 1937[1]	9463 ± 1135[2]	< 0.001
Neutrophils	2913 ± 794[1,2]	6056 ± 1782[1]	6852 ± 1135[2]	< 0.001
Lymphocytes	1960 ± 457	2222 ± 582[3]	1803 ± 508[3]	< 0.01
Monocytes	490 ± 137[1,2]	691 ± 220[1]	577 ± 127[2]	< 0.01
T cells	701 ± 50[11]	1248 ± 507[1,3]	546 ± 462[3]	< 0.05
CD4+ T cells	435 ± 364	722 ± 304[3]	300 ± 279[3]	< 0.001
N	283 ± 280	430 ± 230[3]	177 ± 184[3]	< 0.001
CM	53 ± 35[1]	108 ± 47[1,3]	42 ± 32[3]	< 0.05
EM	92 ± 52	172 ± 135	76 ± 61	> 0.05
TEMRA	7 ± 5	12 ± 6[3]	5 ± 6[3]	< 0.05
CD8+ T cells	183 ± 140[1]	348 ± 165[1,3]	139 ± 107[3]	< 0.01
N	98 ± 75[1]	171 ± 95[1,3]	70 ± 48[3]	< 0.05
CM	7 ± 7	11 ± 7[3]	5 ± 4[3]	< 0.05
EM	63 ± 59[1]	116 ± 77[1,3]	49 ± 51[3]	< 0.05
TEMRA	15 ± 14[1]	50 ± 44[1,3]	15 ± 15[3]	< 0.05

Data displayed as mean ± SD. p > 0.05 indicates no significant differences between trials.
[1], significant difference between Pre-Ex and Post-Ex ($P < 0.05$)
[2], significant difference between Pre-Ex and Rec-Ex ($P < 0.05$)
[3], significant difference between Post-Ex and Rec-Ex ($P < 0.05$)

Abbreviations: Pre-Ex, pre-exercise; Post-Ex, post-exercise; Rec-Ex, Recovery-Exercise; WBC, white blood cell; N, Naïve; CM, central memory; EM, effector memory; TEMRA, terminally differentiated effector memory.

Mitochondrial Membrane Potential

ΔΨm for each T cell populations at Pre-Ex are reported in Supplementary Figure 2, expressed as a ratio of the raw MSO-MFI to the BAM-15 treated control. There were no differences between total lymphocytes, $CD3^+$, $CD4^+$ and $CD8^+$ T cells. Within $CD4^+$ T cells, EM exhibited greater ΔΨm than naïve cells ($p = 0.03$) and within $CD8^+$ T cells, ΔΨm was greater in CM than naïve cells ($p = 0.01$).

Relative changes in total $CD4^+$ and $CD8^+$ T cell ΔΨm (and associated daughter subpopulations) in response to prolonged cycling are shown in Figure 2A-D. There were no changes in ΔΨm between Pre-Ex and Post-Ex, except for an increase in naïve $CD8^+$ T cells ($p = 0.02$, Figure 2D). In Recovery relative to Pre-Ex, ΔΨm increased in total $CD3^+$ T cells ($p = 0.002$, Figure 2B), and this change was driven by increases within $CD8^+$ CM ($p = 0.03$), $CD4^+$ CM ($p = 0.02$) and EM ($p = 0.008$) subsets (Figures 2C-D). For absolute changes, there was an increase in ΔΨm of $CD4^+$ T cells between Pre-Ex and Recovery only ($p = 0.01$), driven by CM ($p = 0.03$) and EM ($p = 0.01$) subsets (Supplementary Figure 3B). There were no changes in mitochondrial mass in any cell type across any timepoint ($p > 0.05$).

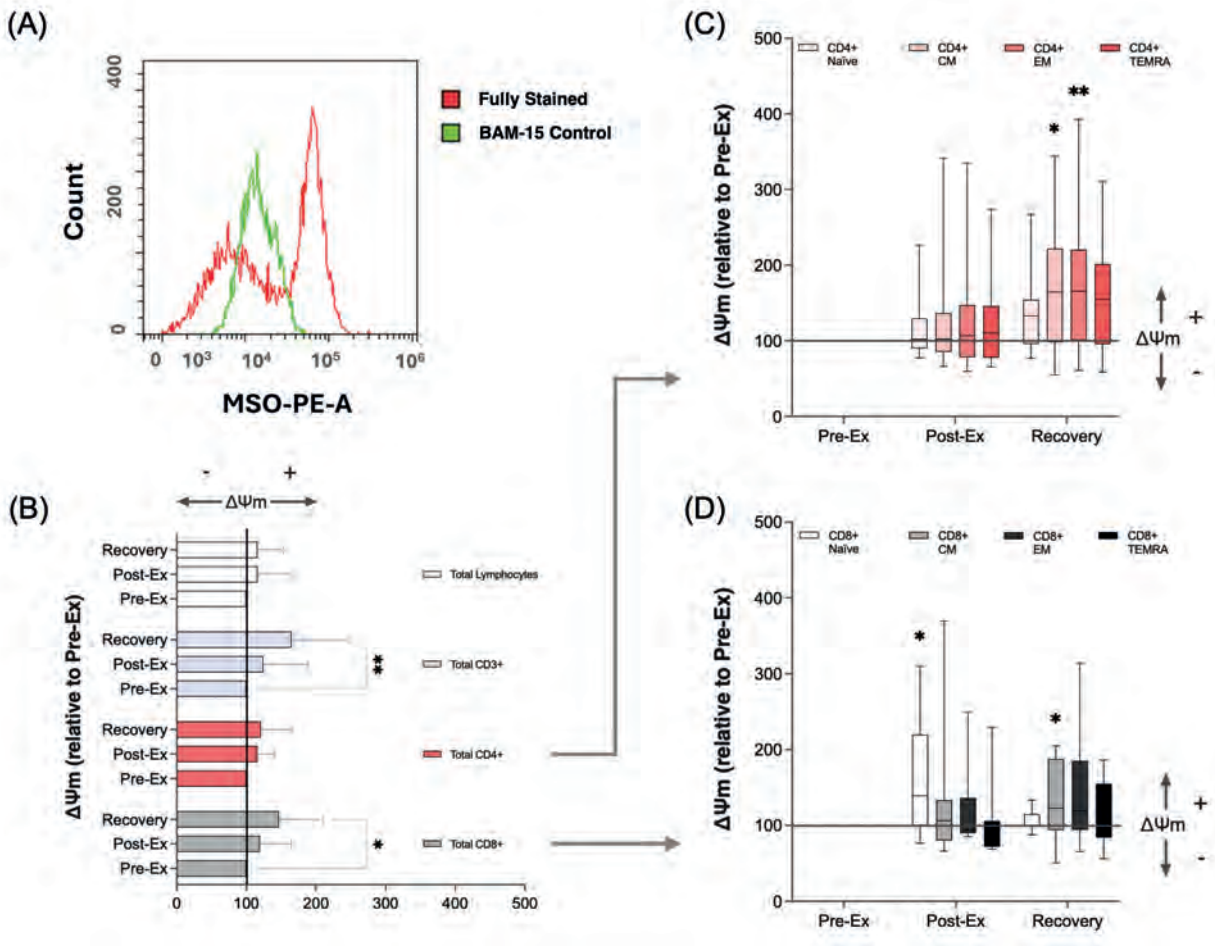


Figure 2. Relative changes in the mitochondrial membrane potential of T Cell subsets in response to prolonged cycling. ΔΨm was expressed as a ratio of the raw mean fluorescence intensity (MFI) of MitoSpy Orange (MSO) to the BAM-15 treated control. (A) Representative histograms depicting MSO-MFI of gated central memory CD4⁺ T cells treated with MSO (red) and BAM-15 + MSO (green). Changes in ΔΨm immediately and in recovery from cycling were expressed relative to Pre-Ex (Increase vs. Pre-Ex >100, Decrease vs. Pre-Ex <100) in total lymphocytes (light grey), CD3⁺ (purple), CD4⁺ (red) and CD8⁺ (dark grey) T cells (B). ΔΨm of naïve, CM, EM and TEMRA subsets (progressively darker colour representing antigen experience: TEMRA > EM > CM > naive) for (C) CD4+ (red) and (D) CD8⁺ T cells (grey) are also depicted. * represents significant differences between cell subsets: *p < 0.05, **p < 0.01.

Changes in the Metabolic Profile of PBMCs and Isolated Naïve T cells in Response to Prolonged Cycling

Purity of Enriched CD4⁺ and CD8⁺ Naïve T cells

The purity of naïve CD4⁺ and CD8⁺ T cells were confirmed by flow cytometry. Prior to enrichment, naïve (CD45RA⁺ CCR7⁻) CD4⁺ and CD8⁺ T cells composed 67.15% ± 16.08 and 52.12% ± 14.69 of the total T cell fraction respectively. After MACS enrichment, the mean frequencies of naïve CD4⁺ and CD8⁺ T cells were 98.85% ± 1.18 and 99.84% ± 0.14 respectively, indicating purity in line with manufacturer standards (> 95%). Subsequently, absolute (Figure 3) and relative (Figure 4) changes in the metabolic phenotypes of PBMCs and enriched naïve CD4⁺ and CD8⁺ T cells in response to prolonged cycling are presented. Immunometabolic outcomes for naïve CD4⁺ and CD8⁺ T cells are presented as a proportion of the PBMC fraction, based on compositional shifts in lymphocytes and monocytes elicited by cycling (Figure 3C–D). A full description of the latter is reported in Supplementary Materials.

Absolute Changes in Metabolic Parameters

Live-cell absolute measurements of OCR in response to modulators of mitochondrial respiration are presented in Figure 3B and used to calculate parameters of mitochondrial function (Figure 3E–I). There were no significant differences in basal, ATP-linked, maximal respiration, proton leak or spare respiratory capacity in naïve CD4⁺ and CD8⁺ T cells or PBMCs between Pre-Ex, Post-Ex and Recovery ($p > 0.05$). Real-time measurements of glycolytic flux (Figure 3J) and rates of ATP synthesis rates (Figure 3K–L) were not significantly changed in naïve CD4⁺ and CD8⁺ T cells, or PBMCs across timepoints. Across all absolute measurements, OCR, glycolytic PER and rates of ATP synthesis were significantly higher in PBMCs than all naïve T cells ($p < 0.0001$). To assist with data interpretation, 95% confidence intervals of the mean differences, effect sizes (Cohen's d) and F-statistics are presented in Supplementary Tables 1 and 2. There were consistent trends for an increase in all metabolic variables between Pre-Ex and Post-Ex for all cell subsets, demonstrating small (0.2 - 0.5) to moderate (0.5 – 0.8) effect sizes. Furthermore, there were consistent trends for a decrease in the same variables between Pre-Ex and Recovery, demonstrating moderate (0.5 – 0.8) to large effect sizes (> 0.8). However, the large confidence intervals indicate variable responses between participants.

Relative Changes in Metabolic Parameters

Measurements of OCR and PER in response to modulators of mitochondrial respiration were used to calculate the relative contributions of mitochondrial parameters within each cell fraction (Figure

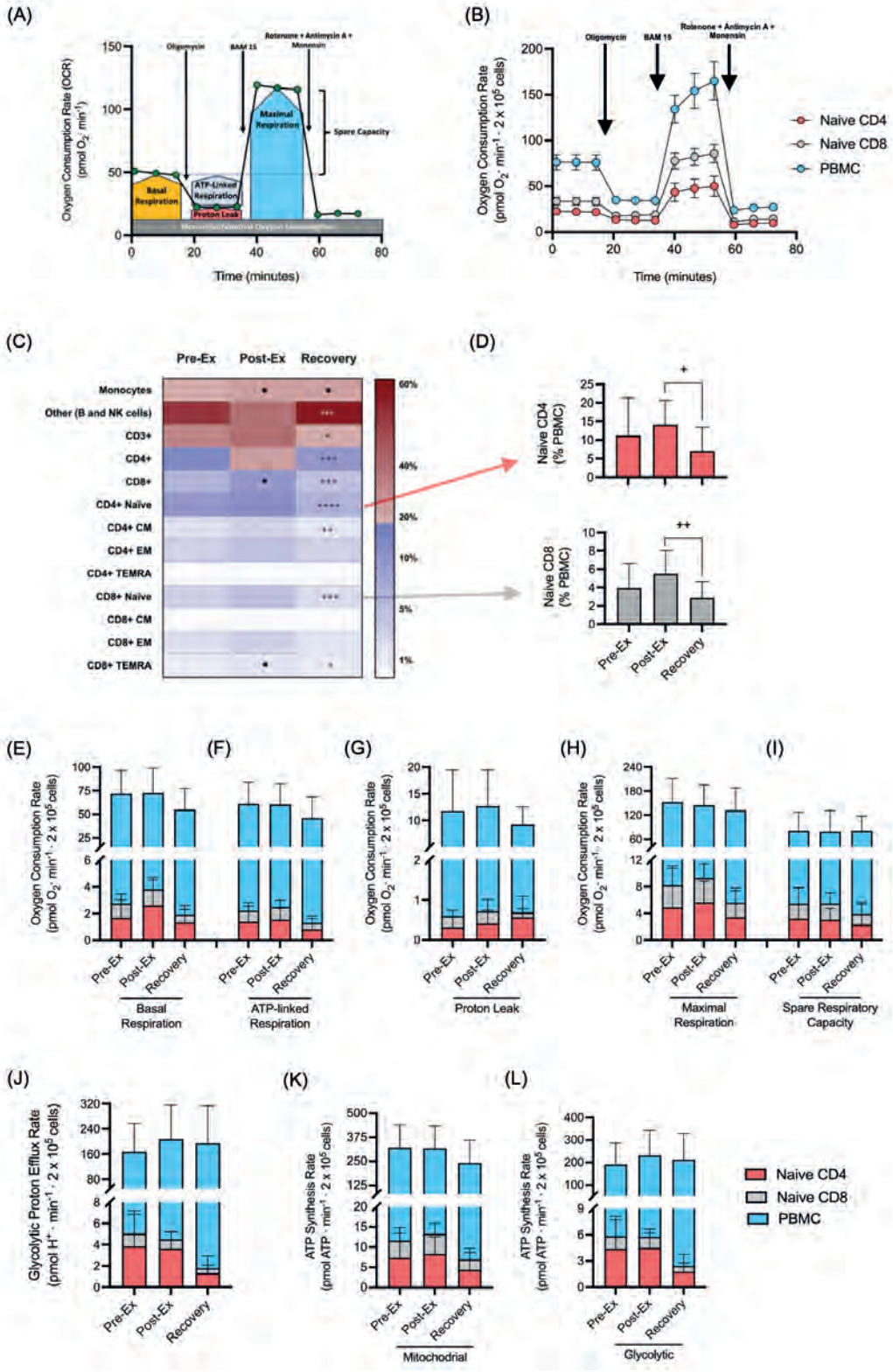


Figure 3. Mitochondrial profile of naïve CD4⁺ and CD8⁺ T cells within 200 x 10^3 seeded PBMCs/well. (A) Schematic representation of changes in oxygen consumption rate (OCR) monitored using a Seahorse XFe96 Analyzer when oligomycin, BAM 15 and rotenone + antimycin A + monensin were injected. Basal (yellow), ATP-linked (grey), maximal respiration (blue), proton leak (red), and spare respiratory capacity (blue) were calculated. (B) Representative live traces of OCR within naïve CD4⁺ T cells (red circles), CD8⁺ T cells (grey circles) and PBMCs (blue circles). OCR was measured continuously throughout the experimental period at baseline followed by the addition of the 3 indicated drugs. (C) A heat map presents the proportions of immune cell populations determined using flow cytometry within Pre-Ex, Post-Ex and Recovery PBMC samples. N.B. monocyte, B cell and NK cell frequencies were calculated from negative populations acquired during flow cytometry analysis and not directly using antibody conjugates. (D) Frequencies of naïve CD4⁺ and CD8⁺ T cells in seeded PBMCs from each timepoint for OCR measurement are graphically depicted. (E) Basal, (F) ATP-linked respiration, (G) Proton leak, (H) Maximal respiration, and (I) Spare respiratory capacity (J) Glycolytic PER, (K) Mitochondrial and (L) Glycolytic ATP production rates are presented for naïve CD4⁺ T cells (red stacked bars), CD8⁺ T cells (grey stacked bars) and PBMCs (total bar). N.B. Blue stacked bars represent values for 'PBMC – naïve CD4⁺ and CD8⁺ T cells'. Data presented as the mean ± SD of 10 participants x 3 timepoints. * indicates significant differences between Pre-Ex and Post-Ex or Recover, and + indicates significant differences between Post-Ex and Recovery: $p > 0.05$, *$p < 0.05$, **$p < 0.01$, +$p < 0.05$, ++$p < 0.01$, +++$p < 0.001$, ++++$p < 0.0001$. All immunometabolism outcomes were significantly greater in PBMCs vs. naïve T cells, but not indicated on every graph.

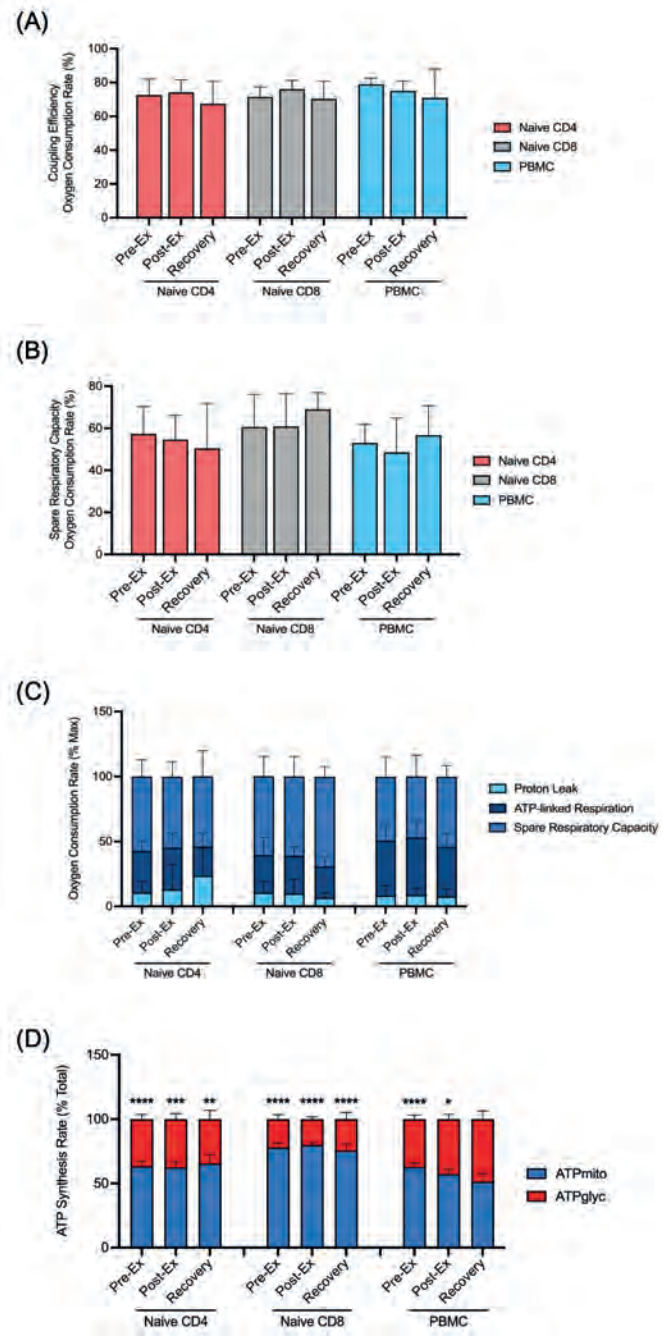


Figure 4. Relative changes in metabolic parameters (% maximal OCR) within isolated naïve CD4⁺ and CD8⁺ T cells vs. PBMCs in response to prolonged cycling. (A) Coupling Efficiency, (B) Spare Respiratory Capacity, (C) Differences in proton leak, ATP-linked respiration, and spare respiratory capacity and (D) ATP synthesis rate (% total). Data presented as the mean ± SD of 10 participants. * indicates significant differences between ATPmito and ATPglyc: $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

4). There were no significant differences in coupling efficiency (% basal OCR, Figure 4A) or spare respiratory capacity (% maximal OCR, Figure 4B) across timepoints ($p > 0.05$). Similarly, there were no changes observed in the proportional contributions of proton leak, ATP-leaked respiration and spare respiratory capacity (Figure 4C) in any cell type or between timepoints ($p > 0.05$). When comparing the relative contribution of glycolysis and mitochondrial respiration to ATP synthesis rate (Figure 4D), total ATP production was driven by mitochondrial respiration relative to glycolysis across all cell types ($p < 0.0001$), with the contribution greater in naïve T cells vs. PBMCs ($p = 0.0004$). This pattern was present across all timepoints, except PBMCs at recovery (% contribution, Pre-Ex: 62.8 ± 9.73, Post-Ex: 57.3 ± 11.88, and Recovery: 51.3 ± 20.27, $p = 0.29$). Although this indicates a shift in metabolic energy phenotype at this timepoint, there were no differences in mitochondrial or glycolytic driven ATP production between timepoints for naïve CD4⁺ ($p = 0.87$), naïve CD8⁺ ($p = 0.61$) T cells or PBMCs ($p = 0.29$). Tabular data, including 95% confidence intervals, effect sizes (Cohen's d) and F-statistics are presented in Supplementary Tables 2 and 3.

Effect of Prolonged Cycling on *Ex vivo* T cell Metabolic Profile upon Activation

Absolute Changes in Metabolic Parameters upon Stimulation

To determine real-time metabolic responses to T cell activation in collected blood samples, enriched naïve T cells and PBMCs were incubated with a CD3/CD28 activator. All samples were seeded at 2 x 105 cells per well, thus enriched naïve CD4⁺ and CD8⁺ T cell numbers were equal across Pre-Ex, Post-Ex and Recovery. However as expected, there were significant differences in the number of T cell subsets within the PBMC fraction across timepoints (Time x Cell subset Interaction: $F_{24,234} = 6.78$, $p < 0.0001$). These differences are graphically depicted in Figure 5A and a full description of the statistics given in Supplementary Tables 4 and 5.

In response to activation, there were significant increases in glycolytic PER (Figure 5F–H) and Supplementary Table 4) in all cell types at all timepoints and significant increases in glycolytic ATP synthesis rate, but not mitochondrial, across all timepoints for naïve CD4⁺ and Naïve CD8⁺ T cells but not PBMCs (Figure 5L-N and Supplementary Table 5). Across all timepoints, absolute PER was significantly greater in PBMCs (average pmol $H^+ \cdot min^{-1}$: 267.84 ± 129.41) vs. naïve CD4⁺ (average pmol $H^+ \cdot min^{-1}$: 70.01 ± 32.20) and CD8⁺ (average pmol $H^+ \cdot min^{-1}$: 41.49 ± 23.55) T cells ($F_{2,54} = 79.67$, $p < 0.0001$, Figure 5F-H). There were no exercise effects found for any variable from any cell type as values remained unaltered between timepoints ($p > 0.05$).

Prolonged Ex Vivo T-cell Stimulation

Two further indicators of naïve T cell activation upon *ex vivo* stimulation are increases in cell diameter (74), and secretion of IL-2, measured in the cell supernatant (10). In response to 12-hours of CD3/CD28 activation, the mean diameter of naïve CD4⁺ (µm, control: 6.37 ± 0.49 vs. activation: 7.50 ± 0.53, Main Effect of Condition: $p < 0.0001$) and CD8⁺ (µm, control: 6.05 ± 0.59 vs. activation: 7.95 ± 0.54, Main Effect of Condition: $p < 0.0001$) T cells significantly increased across all timepoints (Figure 6A–B), but there were no differences between timepoints ($p = 0.13$). The concentration of IL-2 (pg/mL) measured in the supernatant isolated from naïve CD4⁺ and naïve CD8⁺ T cells did not change after activation across all timepoints, ($F_{2,20} = 0.29$, $p = 0.75$ and $F_{2,29} = 0.20$, $p = 0.82$) (Figure 6C–D). There were no significant differences across timepoints ($p = 0.18$) and no correlation between changes in IL-2 concentration and PER in any cell type (Figure 6E–F).

Effect of Prolonged Cycling on Metabolic Substrates

To evaluate changes in circulating nutrient availability in response to prolonged moderate-intensity cycling, the concentrations of lactate, glucose, glutamine, and triglycerides were quantified (Figure 7A–C). There was no significant increase in lactate concentration

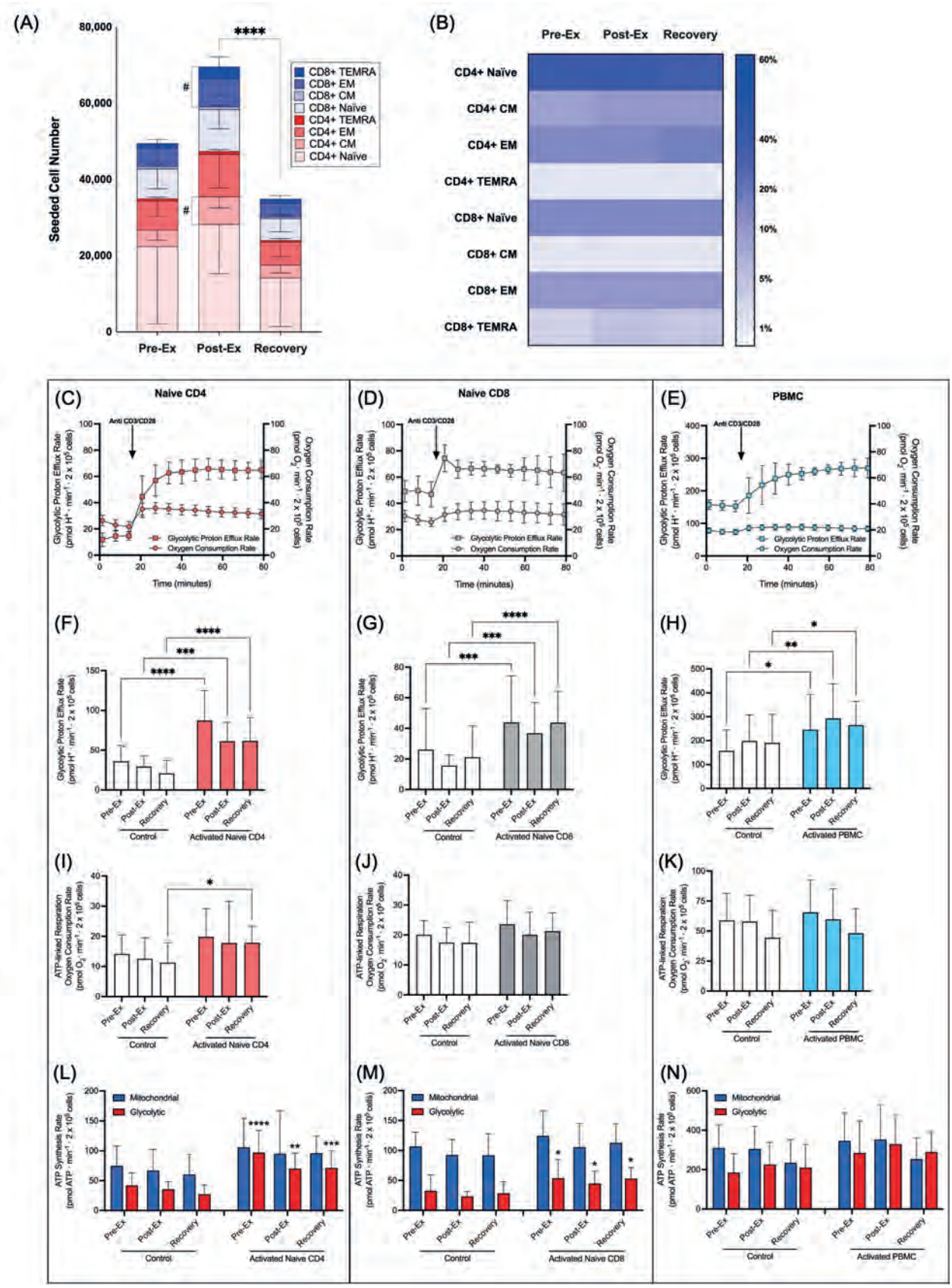


Figure 5. Real-time metabolic responses to CD3/CD28 activation within enriched naïve CD4⁺ and naïve CD8⁺ T cells, and PBMCs. (A) a stacked graph presents the numbers and (B) a heat map shows the frequency of T cell subsets within the seeded PBMC fraction for the activation assay. Representative traces of Glycolytic PER vs. Mitochondrial OCR upon activation of (C) Naïve CD4⁺, (D) Naïve CD8⁺ T cells, and (E) PBMCs were recorded with a Seahorse XFe96 Analyzer. CD3/CD28 activation beads were injected at 14 – 20 minutes, and PER was measured continuously throughout the experimental period after 3 measurements at baseline. (F–H) PER and (I–K) ATP-linked respiration of activated naïve CD4⁺ (red bars), naïve CD8⁺ (grey bars) T cells, and PBMCs (blue bars) vs. control (white bars) were then calculated. Differences in ATP synthesis rate between mitochondrial respiration (blue bars) and glycolysis (red bars) on (L) Naïve CD4⁺, (M) Naïve CD8⁺ T cells, and (N) PBMC. Data presented as the mean ± SD of 10 participants. # indicates significant differences between Pre-Ex and Post-Ex, and * indicates significant differences between timepoints or condition: $p > 0.05$, #$p < 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

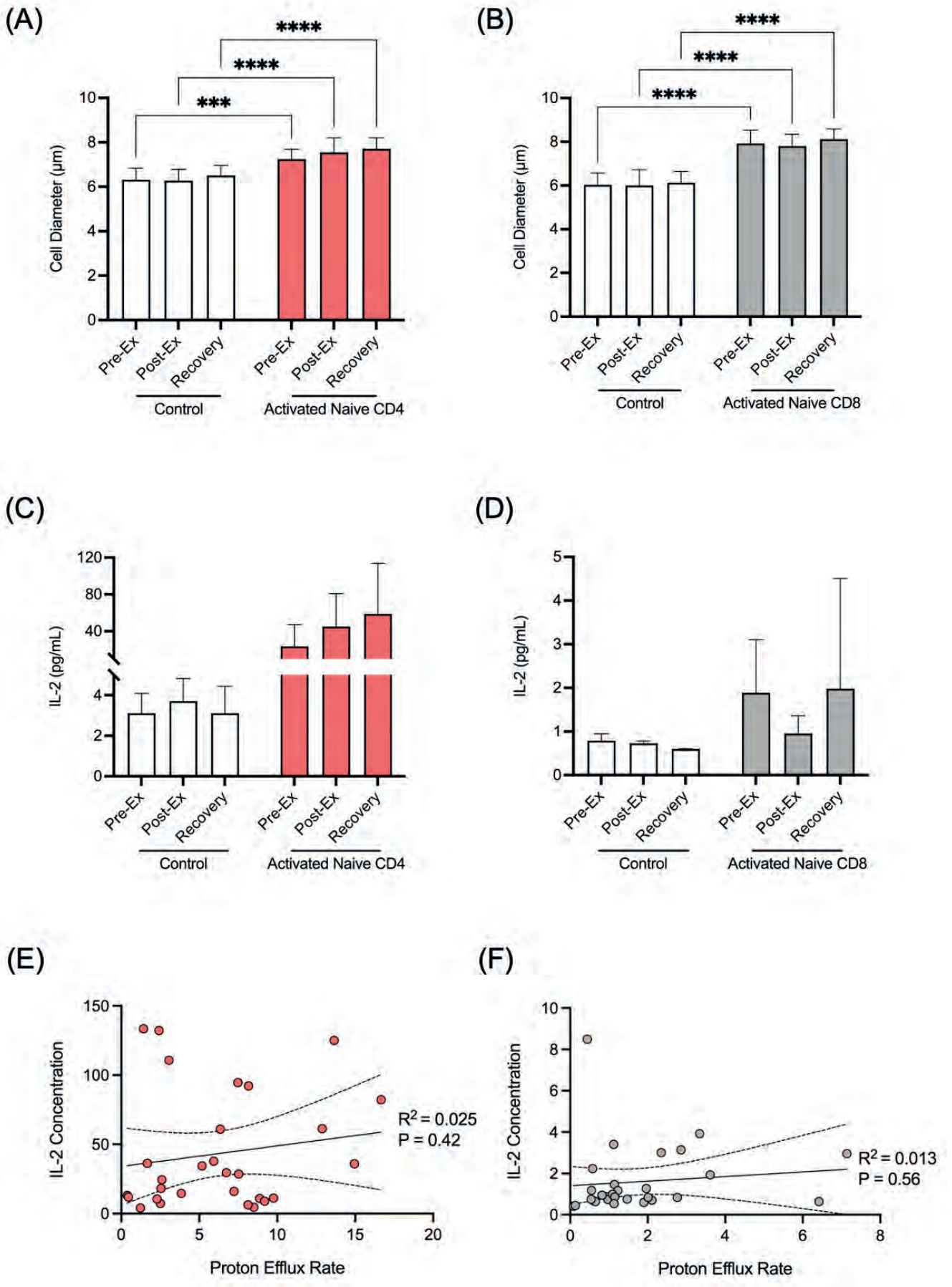


Figure 6. (A–B) Changes in cell diameter and (C–D) IL-2 secretion from naïve CD4⁺ T cells (red bars) and naïve CD8⁺ T cells (grey bars) after prolonged activation or control (white bars). A Pearson correlation between IL-2 concentration and PER are then indicated for (E) naïve CD4⁺ T cells (red circles) and (F) naïve CD8⁺ T cells (grey circles). Data presented as the mean ± SD of 10 participants. * indicates significant differences between control and activation: $p > 0.05$, ***$p < 0.001$, ****$p < 0.0001$.

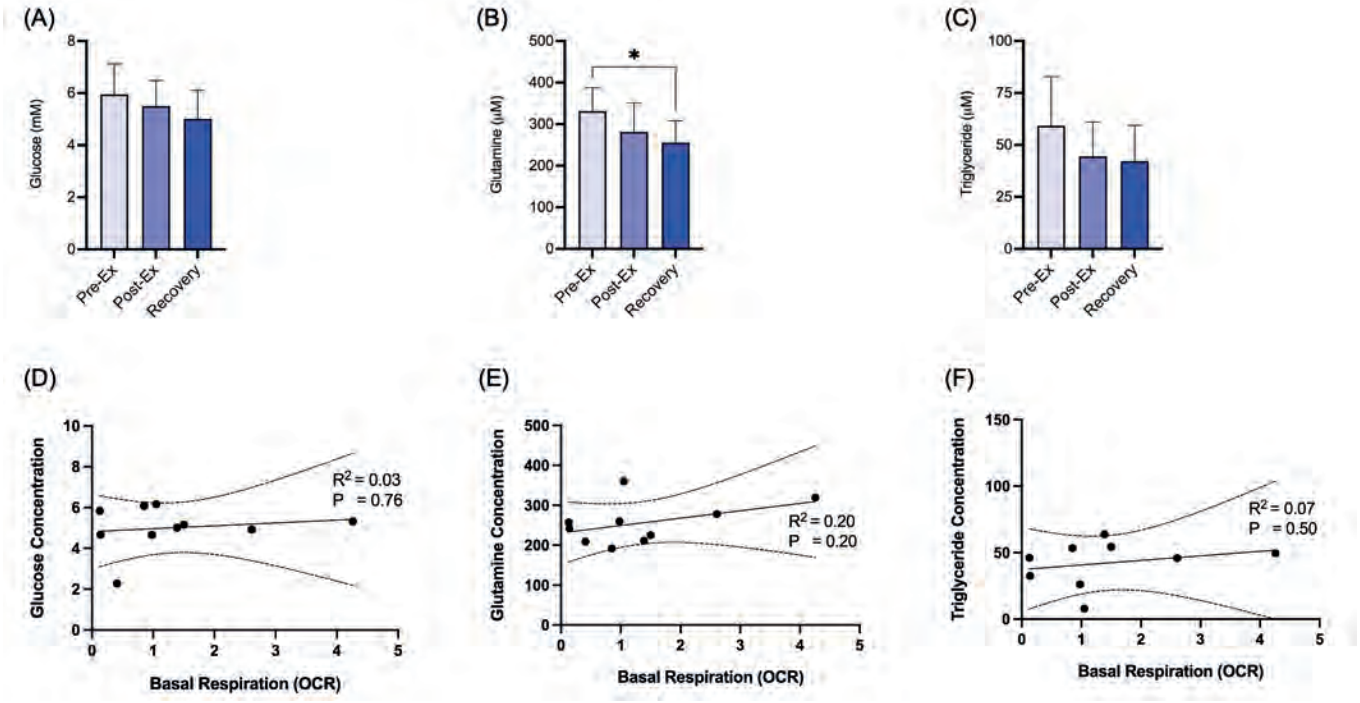


Figure 7. Concentrations of (A) glucose, (B) glutamine, and (C) triglycerides in response to prolonged cycling. (D–F) Representative correlations between each metabolite and basal respiration are provided. Data presented as the mean ± SD of 10 participants. * indicates significant differences between timepoints: $p > 0.05$, $*p < 0.05$.

from Rest (Pre-Ex: 1.83 ± 0.54 mmol/L) to the prescribed LT-1 exercise intensity domain (Average during exercise: 2.09 ± 0.39 mmol/L, $p = 0.25$). There were no differences in glucose ($F_{2,27} = 1.89$, $p = 0.17$) and triglyceride ($F_{2,25} = 2.17$, $p = 0.14$) concentrations across timepoints, but glutamine concentration (Figure 7B) significantly decreased at Recovery compared to Pre-Ex ($F_{2,27} = 4.17$, $p = 0.03$). There were no significant correlations between changes in plasma glucose, glutamine or triglyceride concentration and metabolic outcomes in all cell types across any timepoints.

DISCUSSION

This study investigated changes to the bioenergetic profile of T cells in response to prolonged moderate intensity cycling. The primary finding was that relative to rest, 2 hours of cycling at 95% LT1 elicited no marked changes to the immunometabolic profiles of naïve $CD4^+$ and $CD8^+$ T cells, or PBMCs immediately after and 2 hours into recovery. Using XF analysis, absolute and relative measures of mitochondrial respiration, glycolytic flux and ATP synthesis rate were similar across all timepoints. The contribution of mitochondrial respiration to ATP production was greater than glycolysis in naïve T cells across all timepoints and Pre-Ex and Post-Ex in PBMCs, but not Recovery. Using flow cytometry to further examine the mitochondrial activity of each T cell subset, the $\Delta\Psi m$ of $CD8^+$ and memory $CD4^+$ T cells was observed to be greater in Recovery vs. Rest. Functional T cell responses were preserved across all timepoints, with no changes in cellular bioenergetic responses after *ex vivo* CD3/CD28 activation in all cell fractions. Collectively, these data indicate that the metabolic phenotype and *ex vivo* responses to activation of the total PBMC fraction and isolated naïve T cells were largely unaltered within 2 hours of prolonged moderate intensity cycling.

The current study demonstrated an expected exercise-induced lymphocytosis (7, 23, 62, 79), with significantly greater concentrations of CM $CD4^+$ (+204%), and naïve (+174%), EM (+184%) and TEMRA $CD8^+$ T cells (+333%) in peripheral blood immediately after prolonged cycling at 95% LT1, relative to rest (Table 5). A pattern of preferential mobilisation of antigen experienced T cells was present for $CD8^+$ (TEMRA > EM > N), but not $CD4^+$ T cells. The mobilisation of antigen experienced T cells during bouts of exercise has been a reproducible finding in the field of exercise immunology (7, 29, 82) and relates to higher cell surface expression of beta-2 adrenergic receptor (19). The subsequent redeployment of these cells from the circulation is believed to govern immunosurveillance during recovery (37, 62, 67) and shifts in cellular metabolism have been proposed to facilitate this (3, 23, 34, 51, 72). Data from the present study indicate no marked changes in the bioenergetic profile of the total PBMC fraction immediately after prolonged moderate intensity cycling. This corroborates previous data indicating limited effect of moderate-to-vigorous intensity cycling (30 minutes at 65–70% $\dot{V}O_{2max}$) (75) or maximal swimming (72) on the mitochondrial respiratory function of PBMCs on a cell-by-cell basis. Interestingly, low-intensity cycling (~35% $\dot{V}O_{2peak}$) for 1 hour has been reported to increase fatty acid-dependent respiration in PBMCs (41), indicating metabolic sensitivity of PBMCs to acute exercise. When contrasting the current study design to Liepinsh et al, 2020, participants in our study were more aerobically trained ($\dot{V}O_{2max}$: 53.9 ± vs 33.3 ± 1.3), exercised at a substantially higher relative exercise intensity ($\dot{V}O_{2max}$: 66.1 ± 11.1 vs. 36.0 ± 1.8) and this resulted in less contribution of fat oxidation (Fat: Carbohydrate Oxidation Ratio: 0.22 ± 0.44 vs. 0.59 ± 0.43). Examination of fatty acid specific respiration (41) vs. real-time measurements of mitochondrial and glycolytic energy metabolism and carbohydrate specific respiration in other studies (72, 75) in part, explain

the observed inverse relationship between exercise intensity and PBMC respiration. However, lower intensity exercise would have perturbed peripheral blood immune composition to a lesser degree than in the present (Table 5, Figure 3C) and previous studies (72, 75). Examining cell-by-cell changes after more intense bouts of exercise, where leukocyte ingress/ egress is more marked and not uniform (23, 55, 62) makes examination of the cells of interest more challenging. Analysis 2 hours into recovery was a novel element of the present study design and our data indicate modulation of energy phenotype within PBMCs at this timepoint. The contribution of mitochondrial respiration to ATP production was significantly greater than glycolysis in PBMCs at rest and immediately after prolonged cycling; however, there was no difference in recovery (Figure 4D). This relative shift favouring greater glycolytic > mitochondrial contribution indicates an activated energy phenotype; however, there was no accompanying absolute changes in PER. Collectively, PBMC bioenergetics were largely unaltered within two hours of prolonged cycling. A conundrum persists when examining functional changes of the PBMC fraction after single bouts of exercise. This was exemplified by the notable compositional shifts in $CD4^+$ and $CD8^+$ N, CM, EM and TEMRA cells after cycling (Table 5, Figure 3C), underpinning the importance of providing single cell resolution on measures of cellular bioenergetics (27).

To overcome these challenges, the present study used immunomagnetic separation to enrich naïve T cells from the PBMC fraction and examine their metabolic phenotype and complemented by a flow cytometry assay that coupled immunophenotyping to measurements of ΔΨm. Rates of basal OCR (Figure 3E–I) and PER (Figure 5F–H) were substantially lower in naïve T cells vs. PBMCs and ΔΨm was lower in naïve vs. EM $CD4^+$ and CM $CD8^+$ T cells (Supplemental Figure 2). This confirms previous findings indicating greater mitochondrial and glycolytic respiration (17) and ΔΨm in antigen experienced vs. naive T cell subsets (73). These differences are independent of any effect of exercise, making interpretation of changes within the mixed PBMC fraction between timepoints challenging. However, similar to PBMCs, there were no statistically significant differences in the real-time metabolic profiles of enriched naïve $CD4^+$ and $CD8^+$ T cells either immediately or 2 hours after cessation of cycling, despite mean trends mirroring naïve T cell mobilisation patterns (supplementary Table 1). Specifically, absolute and relative OCR measures of basal, maximal and ATP-linked respiration, proton leak and spare respiratory capacity were unaltered (Figures 3E-I). Furthermore, rates of glycolysis nor the relative contributions of glycolysis and mitochondrial respiration to ATP synthesis rate significantly changed across timepoints (Figures 3J-L). In contrast, flow cytometry data revealed a relative increase in the ΔΨm of naïve $CD8^+$ T cells immediately after prolonged cycling (+43.0%, Figure 2D). Coupling MSO to immunophenotyping provides a reflection of total mitochondrial activity based on charge, rather than directly quantifying oxygen consumption rate during XF analysis (25). Similar dyes (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide (JC-1)) have been used previously to examine changes in ΔΨm after bouts of exercise; however, measurements were only taken from the entire lymphocyte population based on forward vs. side scatter (78). Perhaps surprisingly, there were no significant changes in ΔΨm within CM, EM or TEMRA $CD4^+$ and $CD8^+$ T cells immediately after cycling (Figure 2C-D). The ΔΨm of these antigen experienced T cells was significantly greater than naive T cells at rest (Supplemental Figure 2) and therefore changes in the $CD8^+$ naïve T cell population may reflect an exercise-responsive increase in mitochondrial activity due to their quiescent state and greater metabolic flexibility compared to antigen experience T cells (69). In recovery, ΔΨm of CM $CD8^+$ (+30.7%) and CM (+66.7%) and EM $CD4^+$ T cells (+75.7%) were significantly elevated vs. Pre-Ex (Figure 2C-D). Mechanisms underpinning these cell specific changes are unclear. Circulating metabolites that support T cell function, namely glucose and triglyceride concentrations remained stable throughout the trial; however, there was a significant decrease in glutamine concentration in Recovery relative to Rest (Figure 7). This is well-documented after prolonged bouts of exercise (53, 59) and may relate to increased uptake by the liver to support acute phase protein production or notably, uptake from circulating active lymphocytes (22). It is well established that lymphocytes utilise glutamine during periods of stress (i.e., exercise) to provide energy for biosynthesis and to support cell proliferation (84). Although immune cell bioenergetics evaluated via EFA were largely resistant to the drop in plasma glutamine, we can speculatively suggest that the uptake of glutamine from the circulation may, in part, explain elevated ΔΨm of T cell memory subsets in recovery (15, 39).

To determine the impact of prolonged cycling on functional metabolic outcomes in naïve and PBMCs (naive and antigen experienced T cells - CM, EM and TEMRA), real-time metabolic responses to activation were examined *ex vivo* using a CD3/CD28 activator (Figure 5C–E). This approach enabled cellular bioenergetics to be profiled in real-time from activated T-cells within the PBMC fraction and enriched naïve T cells. In response to *ex vivo* activation, there was an increase in maximal glycolytic flux defined by PER, cell diameter, but not IL-2 production (Figures 5 and 6); however, there were no differences observed between timepoints, indicating that prolonged exercise didn't modulate T cell activation responses. Recent data indicates elevated activation-induced proliferation responses of $CD3^+$ T cells after high vs. moderate intensity exercise which may partly explain the lack of immunometabolic differences across timepoints (65). Further, a study by Withnall et al, 2024 indicated lower energetic demand and cytokine production from activated T cells (using PMA and ionomycin) isolated from physically active vs. inactive individuals (83). Our study cohort of aerobically trained participants may therefore demonstrate less sensitivity to metabolic reprogramming (via changes in PER) in T cells after prolonged cycling. This is challenging to interpret without a direct comparison with a sedentary control group.

FUTURE DIRECTIONS & LIMITATIONS

Collectively, these data indicate that bioenergetic profiles and metabolic responses to activation of naïve $CD4^+$ and $CD8^+$ T cells and the total PBMC fraction were largely unaltered in response to prolonged moderate intensity cycling. These analyses included measurements both immediately and 2 hours into recovery under controlled laboratory conditions, whereby nutrition (for 12 hours) and rest were controlled. It is noteworthy that recent studies employing single cell RNA sequencing have revealed pro-gly-

colytic shifts within mobilised EM T cells after bouts of exhaustive exercise (3), independent of shifts in cell composition. This study directly examined naïve T cells, due to their high proportion within the PBMC fraction and ease of isolation compared to antigen experienced T cells which are at a lower frequency (CM, EM, and TEMRA, Figure 3C). Although significant mobilisation of $CD8^+$ naïve T cells was reported in the present study, the preferential mobilisation of TEMRA > EM > CM > N (7, 29) highlights that comprehensive examination of antigen experienced T cell bioenergetics, and other cell types (e.g., NK cells, monocytes and B cells) after exercise bouts of different intensity is warranted. Our flow cytometry data provided some insight, revealing that mobilisation of antigen-experienced T cells was not accompanied by changes in $\Delta\Psi m$. Mitochondrial activity was higher in T memory cells in recovery (Figure 2) and this finding warrants further investigation.

Given the emerging literature in support of immune cell bioenergetic adaptions after exercise training (2, 32) it is important to highlight that our findings are restricted to aerobically trained males and females. Most participants in the current study would be classified as having 'Excellent' aerobic fitness based on their age category ($mL \cdot kg^{-1} \cdot min^{-1}$: male > 57.1, female > 46.5), as defined by American College of Sports Medicine (60). Our sample size was relatively small due to the logistical constraints of conducting extensive and detailed biological analysis. This prevented further stratification of data to examine sex-specific differences in acute bioenergetic responses to exercise. Recent studies have highlighted differences between males and females with regards to immunity and exercise (4, 14), although our initial data (N=5 vs. N=5) revealed no striking differences (data not shown). The changes in some bioenergetic variables exhibited moderate to large effect sizes and these should be explored in future studies using a larger sample size.

It is important to acknowledge limitations surrounding the examination of immunometabolic responses to bouts of exercise in humans. We adopted a study design that mitigated time spent processing peripheral blood samples to robustly examine immunometabolic responses in single T cell subsets. However, the time taken to collect, purify and analyse samples (≈ 3 hours) may have influenced cells in vivo metabolic state, despite the rigorous control measures employed. All cells were washed into standardised glucose rich media (i.e. RPMI), but future studies could consider autologous serum as a media to better preserve the bioenergetic state of isolated cells *ex vivo* (18).

Many previous studies examining immunometabolic responses to bouts of exercise have mostly defined 'intensity' based on a proportion of $\dot{V}O_{2max}$ (40), which doesn't account for inter-individual variation in metabolic thresholds that occur at different stages of $\dot{V}O_{2max}$ (40). The current study therefore used 95% LT1 to prescribe a metabolically controlled bout of cycling near aerobic threshold. At this intensity, exercise can be sustained for prolonged durations with minimal fatigue and metabolite (e.g. lactate and adrenaline) accumulation (9, 30). An ongoing narrative in exercise immunology literature purports that prolonged arduous exercise (≥ 2 hours) may impair aspects of immune function (8, 48–50, 68). For the current study population of aerobically trained young males and females, 2 hours of cycling at a moderate intensity (66.1 ± 11.1 % $\dot{V}O_{2max}$) was subjectively perceived as 'fairly light' (RPE: 11.1 ± 1.9) and 'fairly good' (affective response: 2.5 ± 0.6) for physical exertion and enjoyment respectively, despite significant energy expenditure (1357 ± 203 kcal). Although not 'arduous', these data indicate that despite robust T cell mobilisation in response to prolonged cycling, bioenergetic responses were unaltered, therefore providing no evidence to indicate impairment of immune function within 2 hours of recovery, most notably in naïve T cells. To further address this question, future studies should utilise these single cell methods to examine immunometabolic outcomes after more intense bouts or periods of exercise training.

CONCLUSION

These data indicate no marked perturbations in naïve $CD4^+$ and $CD8^+$ T cell or PBMC bioenergetics either immediately or 2 hours after cycling. There was an increase in the mitochondrial membrane potential of memory $CD4^+$ and $CD8^+$ T cells 2 hours following cycling and this finding warrants further investigation.

ETHICS STATEMENT

The study was given favourable ethical opinion by the Science, Technology, Engineering and Mathematics ethical committee at the University of Birmingham (ERN_19-1574PA3).

AUTHOR CONTRIBUTIONS

AJW conceptualised the study and provided project direction. JPB, AJW and FP designed Seahorse experiments. AJW, JS, NG and SKD designed $\Delta\Psi m$ flow cytometry assays. FP and JS carried out all data acquisition. GAW and TP provided insight on study design. Data analyses and presentation were carried out by FP, JB and AJW. AJW interpreted the data and drafted the manuscript, with support from FP. All authors undertook the revision and final approval of the manuscript. Artificial Intelligence (AI) was not used in any aspect of the study, writing or otherwise.

FUNDING

This project was supported by a scholarship grant from the Indonesian Endowment Fund for Education awarded to FP and supervised by AJW. Funding was also provided by a grant from the Society for Endocrinology awarded to AJW.

CONFLICT OF INTEREST

None of the authors declare a conflict of interest.

SUPPLEMENTARY MATERIALS

Link to be added.

REFERENCES

1. 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. Medicine and Science in Sports and Exercise 54: 1288–1299, 2022. doi: 10.1249/MSS.0000000000002914.
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. Scientific Reports 12, 2022. doi: 10.1038/s41598-022-11458-4.
3. Batatinha H, Diak DM, Niemiro GM, Baker FL, Smith KA, Zúñiga TM, Mylabathula PL, Seckeler MD, Lau B, LaVoy EC, Gustafson MP, Katsanis E, Simpson RJ. Human lymphocytes mobilized with exercise have an anti-tumor transcriptomic profile and exert enhanced graft-versus-leukemia effects in xenogeneic mice. Frontiers in Immunology 14: 1–14, 2023. doi: 10.3389/fimmu.2023.1067369.
4. Bauza DER, Silveyra P. Sex Differences in Exercise-Induced Effects on Respiratory Infection and Immune Function. .
5. Bisset S. Effect of Muscular Activity prior to Venepuncture on the Respiration of Leucocytes in vitro. 1958.
6. Burke LM, Hawley JA, Wong SHS, Jeukendrup AE. Carbohydrates for training and competition. Journal of Sports Sciences 29, 2011. doi: 10.1080/02640414.2011.585473.
7. Campbell JP, Riddell NE, Burns VE, Turner M, van Zanten JJCSV, Drayson MT, Bosch JA. Acute exercise mobilises $CD8^+$ T lymphocytes exhibiting an effector-memory phenotype. Brain, Behavior, and Immunity 23: 767–775, 2009. doi: 10.1016/j.bbi.2009.02.011.
8. Campbell JP, Turner JE. Debunking the Myth of Exercise-Induced Immune Suppression: Redefining the Impact of Exercise on Immunological Health Across the Lifespan. Frontiers in Immunology 9: 1–21, 2018. doi: 10.3389/fimmu.2018.00648.
9. Cannon DT, White AC, Andriano MF, Kolkhorst FW, Rossiter HB. Skeletal muscle fatigue precedes the slow component of oxygen uptake kinetics during exercise in humans. Journal of Physiology 589: 727–739, 2011. doi: 10.1113/jphysiol.2010.197723.
10. Cantrell DA, Smith KA. The Interleukin-2 T-Cell System: A New Cell Growth Model [Online]. www.sciencemag.org.
11. Carney CE, Buysse DJ, Ancoli-Israel S, Edinger JD, Krystal AD, Lichstein KL, Morin CM. The consensus sleep diary: Standardizing prospective sleep self-monitoring. Sleep 35: 287–302, 2012. doi: 10.5665/sleep.1642.
12. Chapman NM, Shrestha S, Chi H. Metabolism in immune cell differentiation and function. 2017.
13. Cohen J. Statistical Power Analysis for the Behavioral Sciences, 2nd Edition. 1988.
14. Cortes CJ, De Miguel Z. Precision Exercise Medicine: Sex Specific Differences in Immune and CNS Responses to Physical Activity. Brain Plasticity 8: 65–77, 2022. doi: 10.3233/bpl-220139.
15. Cruzat V, Rogero MM, Keane KN, Curi R, Newsholme P. Glutamine: Metabolism and immune function, supplementation and clinical translation. Nutrients 10, 2018. doi: 10.3390/nu10111564.
16. Deyhle MR, Gier AM, Evans KC, Eggett DL, Nelson WB, Parcell AC, Hyldahl RD. Skeletal muscle inflammation following repeated bouts of lengthening contractions in humans. Frontiers in Physiology 6, 2016. doi: 10.3389/fphys.2015.00424.
17. Dimeloe S, Mehling M, Frick C, Loeliger J, Bantug GR, Sauder U, Fischer M, Belle R, Develioglu L, Tay S, Langenkamp A, Hess C. The Immune-Metabolic Basis of Effector Memory $CD4^+$ T Cell Function under Hypoxic Conditions. The Journal of Immunology 196: 106–114, 2016. doi: 10.4049/jimmunol.1501766.
18. Dorneles GP, da Silva IM, Santos MA, Elsner VR, Fonseca SG, Peres A, Romão PRT. Immunoregulation induced by autologous serum collected after acute exercise in obese men: a randomized cross-over trial. Scientific Reports 10: 1–16, 2020. doi: 10.1038/s41598-020-78750-z.
19. Fan X, Wang Y. β2 Adrenergic receptor on T lymphocytes and its clinical implications. Progress in Natural Science 19: 17–23, 2009. doi: 10.1016/j.pnsc.2008.10.001.
20. Field CJ, Gougeon R, Marliss EB. Circulating mononuclear cell numbers and function during intense exercise and recovery. Journal of Applied Physiology 71: 1089–1097, 1991. doi: 10.1152/jappl.1991.71.3.1089.
21. Foster J. The General Practice Physical Activity Questionnaire (GPPAQ) A screening tool to assess adult physical activity levels , within primary care Updated May 2009. Nhs : 1–21, 2009.
22. Gleeson M. The Journal of Nutrition 7th Amino Acid Assessment Workshop Dosing and Efficacy of Glutamine Supplementation in Human Exercise and Sport Training 1,2. 2008.
23. Graff RM, Kunz HE, Agha NH, Baker FL, Laughlin M, Bigley AB, Markofski MM, LaVoy EC, Katsanis E, Bond RA, Bollard CM, Simpson RJ. β2-Adrenergic receptor signaling mediates the preferential mobilization of differentiated subsets of $CD8^+$ T-cells, NK-cells and non-classical monocytes in response to acute exercise in humans. Brain, Behavior, and Immunity 74: 143–153, 2018. doi: 10.1016/j.bbi.2018.08.017.
24. Gubser PM, Bantug GR, Razik L, Fischer M, Dimeloe S, Hoenger G, Durovic B, Jauch A, Hess C. Rapid effector function of memory $CD8^+$ T cells requires an immediate-early glycolytic switch. Nature Immunology 14: 1064–1072, 2013. doi: 10.1038/ni.2687.
25. Gudgeon N, Munford H, Bishop EL, Hill J, Fulton-Ward T, Bending D, Roberts J, Tennant DA, Dimeloe S. Succinate uptake by T cells suppresses their effector function via inhibition of mitochondrial glucose oxidation. Cell Reports 40, 2022. doi: 10.1016/j.celrep.2022.111193.
26. Hardy CJ, Rejeski WJ. Not What, but How One Feels: The Measurement of Affect during Exercise. Journal of Sport and Exercise Psychology 11: 304–317, 2016. doi: 10.1123/jsep.11.3.304.
27. Hodgman CF, Hunt RM, Crane JC, Elzayat MT, LaVoy E. A Scoping Review on the Effects of Physical Exercise and Fitness on Peripheral Leukocyte Energy Metabolism in Humans. 29: 54–87, 2023. doi: 10.1136/bmj.n71.
28. Howley ET, Bassett Jr DR, Welch HG. Criteria for maximal oxygen uptake: review and commentary. Medicine & Science in Sports & Exercise, 27(9), 27: 1292-1301.-1292-1301., 1995.
29. 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. Frontiers in Sports and Active Living 5, 2023. doi: 10.3389/fspor.2023.1120454.
30. Iannetta D, Inglis EC, Mattu AT, Fontana FY, Pogliaghi S, Keir DA, Murias JM. A Critical Evaluation of Current Methods for Exercise Prescription in Women and Men. Medicine and Science in Sports and Exercise 52: 466–473, 2020. doi: 10.1249/MSS.0000000000002147.
31. Jamnick NA, Pettitt RW, Granata C, Pyne DB, Bishop DJ. An Examination and Critique of Current Methods to Determine Exercise Intensity. Sports Medicine 50: 1729–1756, 2020. doi: 10.1007/s40279-020-01322-8.

32. 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. American Journal of Physiology - Endocrinology and Metabolism 322, 2022. doi: 10.1152/AJPENDO.00365.2021.
33. Jeukendrup AE, Wallis GA. Measurement of Substrate Oxidation During Exercise by Means of Gas Exchange Measurements [Online]. doi: 10.1055/s-2004-830512.
34. Jones N, Piasecka J, Bryant AH, Jones RH, Skibinski DOF, Francis NJ, Thornton CA. Bioenergetic analysis of human peripheral blood mononuclear cells. Clinical and Experimental Immunology 182: 69–80, 2015. doi: 10.1111/cei.12662.
35. Jones N, Cronin JG, Dolton G, Panetti S, Schauenburg AJ, Galloway SAE, Sewell AK, Cole DK, Thornton CA, Francis NJ. Metabolic adaptation of human $CD4^+$ and $CD8^+$ T-cells to T-cell receptor-mediated stimulation. Frontiers in Immunology 8, 2017. doi: 10.3389/fimmu.2017.01516.
36. Keir DA, Iannetta D, Mattioni Maturana F, Kowalchuk JM, Murias JM. Identification of Non-Invasive Exercise Thresholds: Methods, Strategies, and an Online App. Sports Medicine 52: 237–255, 2022. doi: 10.1007/s40279-021-01581-z.
37. Krüger K, Lechtermann A, Fobker M, Völker K, Mooren FC. Exercise-induced redistribution of T lymphocytes is regulated by adrenergic mechanisms. Brain, Behavior, and Immunity 22: 324–338, 2008. doi: 10.1016/j.bbi.2007.08.008.
38. La Rocca C, Carbone F, De Rosa V, Colamatteo A, Galgani M, Perna F, Lanzillo R, Brescia Morra V, Orefice G, Cerillo I, Florio C, Maniscalco GT, Salvetti M, Centonze D, Uccelli A, Longobardi S, Visconti A, Matarese G. Immunometabolic profiling of T cells from patients with relapsing-remitting multiple sclerosis reveals an impairment in glycolysis and mitochondrial respiration. Metabolism: Clinical and Experimental 77: 39–46, 2017. doi: 10.1016/j.metabol.2017.08.011.
39. Lai AG, Forde D, Chang WH, Yuan F, Zhuang X, Rubio CO, Song CX, McKeating JA. Glucose and glutamine availability regulate HepG2 transcriptional responses to low oxygen . Wellcome Open Research 3, 2018. doi: 10.12688/wellcomeopenres.14839.1.
40. Lei TH, Wang IL, Chen YM, Liu XH, Fujii N, Koga S, Perry B, Mundel T, Wang F, Cao Y, Dobashi K, Kondo N, Li HY, Goulding RP. Critical power is a key threshold determining the magnitude of post-exercise hypotension in non-hypertensive young males. Experimental Physiology 108: 1409–1421, 2023. doi: 10.1113/EP091429.
41. 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. Physiological Reports 8, 2020. doi: 10.14814/phy2.14489.
42. Lin ML, Hsu CC, Fu TC, Lin YT, Huang YC, Wang JS. Exercise Training Improves Mitochondrial Bioenergetics of Natural Killer Cells. Medicine and Science in Sports and Exercise 54: 751–760, 2022. doi: 10.1249/MSS.0000000000002842.
43. Lovakov A, Agadullina ER. Empirically derived guidelines for effect size interpretation in social psychology. European Journal of Social Psychology 51: 485–504, 2021. doi: 10.1002/ejsp.2752.
44. Matomäki P, Kainulainen H, Kyröläinen H. Corrected whole blood biomarkers – the equation of Dill and Costill revisited. Physiological Reports 6: 1–3, 2018. doi: 10.14814/phy2.13749.
45. Mercier-Letondal P, Marton C, Godet Y, Galaine J. Validation of a method evaluating T cell metabolic potential in compliance with ICH Q2 (R1). Journal of Translational Medicine 19, 2021. doi: 10.1186/s12967-020-02672-7.
46. Morgado JP, Matias CN, Reis JF, Curto D, Alves FB, Monteiro CP. The Cellular Composition of the Innate and Adaptive Immune System Is Changed in Blood in Response to Long-Term Swimming Training. Frontiers in Physiology 11: 1–10, 2020. doi: 10.3389/fphys.2020.00471.
47. Nicoli F, Papagno L, Frere JJ, Cabral-Piccin MP, Clave E, Gostick E, Toubert A, Price DA, Caputo A, Appay V. Naïve $CD8^+$ t-cells engage a versatile metabolic program upon activation in humans and differ energetically from memory $CD8^+$ T-cells. Frontiers in Immunology 9: 1–12, 2018. doi: 10.3389/fimmu.2018.02736.
48. Nieman DC, Henson DA, Dumke CL, Lind RH, Shooter LR, Gross SJ. Relationship between salivary IgA secretion and upper respiratory tract infection following a 160-km race. The Journal of sports medicine and physical fitness 46: 158–162, 2006.
49. Nieman DC, Johanssen LM, Lee JW, Arabatzis K. Infectious episodes in runners before and after the Los Angeles Marathon. Journal of Sports Medicine and Physical Fitness 30: 316–328, 1990.
50. Nieman DC. Is infection risk linked to exercise workload? Medicine and Science in Sports and Exercise 32, 2000. doi: 10.1097/00005768-200007001-00005.
51. Nieman DC, Lila MA, Gillitt ND. Immunometabolism: A Multi-Omics Approach to Interpreting the Influence of Exercise and Diet on the Immune System. Annual Review of Food Science and Technology 10: 341–363, 2019. doi: 10.1146/annurev-food-032818-121316.
52. Oertelt-Prigione S. Immunology and the menstrual cycle. Autoimmunity Reviews 11, 2012. doi: 10.1016/j.autrev.2011.11.023.
53. Parry-Billings M, Budgett R, Koutedakis Y, Blomstrand E, Brooks S, Williams C, Calder PC, Pilling S, Baigrie R, Newsholme EA. Plasma amino acid concentrations in the overtraining syndrome: possible effects on the immune system. Medicine and science in sports and exercise 12: 1353–1358, 1992.
54. Perry SW, Norman JP, Barbieri J, Brown EB, Gelbard HA. Mitochondrial membrane potential probes and the proton gradient: A practical usage guide. BioTechniques 50: 98–115, 2011. doi: 10.2144/000113610.
55. Pradana F, Nijjar T, Cox PA, Morgan PT, Podlogar T, Lucas SJE, Drayson MT, Kinsella FAM, Wadley AJ. Brief cycling intervals incrementally increase the number of hematopoietic stem and progenitor cells in human peripheral blood. Frontiers in Physiology 15, 2024. doi: 10.3389/fphys.2024.1327269.
56. Rangel Rivera GO, Knochelmann HM, Dwyer CJ, Smith AS, Wyatt MM, Rivera-Reyes AM, Thaxton JE, Paulos CM. Fundamentals of T Cell Metabolism and Strategies to Enhance Cancer Immunotherapy. Frontiers in Immunology 12, 2021. doi: 10.3389/fimmu.2021.645242.
57. Rathmell JC, Heiden MGV, Harris MH, Frauwirth KA, Thompson CB. In the absence of extrinsic signals, nutrient utilization by lymphocytes is insufficient to maintain either cell size or viability. Molecular Cell 6: 683–692, 2000. doi: 10.1016/S1097-2765(00)00066-6.
58. Reina-Campos M, Scharping NE, Goldrath AW. $CD8^+$ T cell metabolism in infection and cancer. Nature Reviews Immunology 21: 718–738, 2021. doi: 10.1038/s41577-021-00537-8.
59. Rennie MJ, Edwards RH, Krywawych S, Davies CT, Halliday D, Waterlow JC, Millward DJ. Effect of exercise on protein turnover in man. 1981.

60. Riebe D, Ep-c A, Ehrman JK, Liguori G, Magal M. ACSM's guidelines for exercise testing and prescription. 10th ed. Wolters Kluwer Health, 2018.
61. Robert B, Brown EB. Psychophysical bases of perceived exertion. Medicine & Science in Sports & Exercise 14: 377–381, 1982.
62. Rooney BV, Bigley AB, LaVoy EC, Laughlin M, Pedlar C, Simpson RJ. Lymphocytes and monocytes egress peripheral blood within minutes after cessation of steady state exercise: A detailed temporal analysis of leukocyte extravasation. Physiology and Behavior 194: 260–267, 2018. doi: 10.1016/j.physbeh.2018.06.008.
63. Rothschild-Rodriguez D, Causer AJ, Brown FF, Collier-Bain HD, Moore S, Murray J, Turner JE, Campbell JP. The effects of exercise on complement system proteins in humans: a systematic scoping review. 2022.
64. Scharping NE, Menk AV, Moreci RS, Whetstone RD, Dadey RE, Watkins SC, Ferris RL, Delgoffe GM. The Tumor Microenvironment Represses T Cell Mitochondrial Biogenesis to Drive Intratumoral T Cell Metabolic Insufficiency and Dysfunction. Immunity 45: 374–388, 2016. doi: 10.1016/j.immuni.2016.07.009.
65. Siedlik JA, Deckert JA, Dunbar AJ, Bhatta A, Gigliotti NM, Chan MA, Benedict SH, Bubak M, Vardiman JP, Gallagher PM. Acute high-intensity exercise enhances T cell proliferation compared to moderate-intensity exercise. Applied Physiology, Nutrition, and Metabolism 50: 1–12, 2025. doi: 10.1139/apnm-2024-0420.
66. Simpson RJ, Bigley AB, Agha N, Hanley PJ, Bollard CM. Mobilizing immune cells with exercise for cancer immunotherapy. Exercise and Sport Sciences Reviews 45: 163–172, 2017. doi: 10.1249/JES.0000000000000114.
67. Simpson RJ, Boßlau TK, Weyh C, Niemiro GM, Batatinha H, Smith KA, Krüger K. Exercise and adrenergic regulation of immunity. Brain, Behavior, and Immunity 97: 303–318, 2021. doi: 10.1016/j.bbi.2021.07.010.
68. Simpson RJ Campbell, JP, Gleeson, M, Krüger,K, Nieman, DC,. Pyne, DB, Turner, JE, and Walsh, NP. Can exercise affect immune function to increase susceptibility to infection? Journal of applied sport psychology 26: 8–22, 2020.
69. Slack M, Wang T, Wang R. T cell metabolic reprogramming and plasticity. Molecular Immunology 68: 507–512, 2015. doi: 10.1016/j.molimm.2015.07.036.
70. Sottnik JL, Lori JC, Rose BJ, Thamm DH. Glycolysis inhibition by 2-deoxy-d-glucose reverts the metastatic phenotype in vitro and in vivo. Clinical and Experimental Metastasis 28: 865–875, 2011. doi: 10.1007/s10585-011-9417-5.
71. Spielberger CD, Gonzalez-Reigosa F, Martinez-Urrutia A, Natalicio LFS, Natalicio DS. The State-Trait Anxiety Inventory. Revista Interamericana de Psicología/Interamerican Journal of Psychology 5, 2017. doi: 10.30849/rip/ijp.v5i3.
72. Stampley JE, Cho E, Wang H, Theall B, Johannsen NM, Spielmann G, Irving BA. Impact of maximal exercise on immune cell mobilization and bioenergetics. Physiological Reports 11: 1–10, 2023. doi: 10.14814/phy2.15753.
73. Sukumar M, Liu J, Mehta GU, Patel SJ, Roychoudhuri R, Crompton JG, Klebanoff CA, Ji Y, Li P, Yu Z, Whitehill GD, Clever D, Eil RL, Palmer DC, Mitra S, Rao M, Keyvanfar K, Schrump DS, Wang E, Marincola FM, Gattinoni L, Leonard WJ, Muranski P, Finkel T, Restifo NP. Mitochondrial Membrane Potential Identifies Cells with Enhanced Stemness for Cellular Therapy. Cell Metabolism 23: 63–76, 2016. doi: 10.1016/j.cmet.2015.11.002.
74. Tasnim H, Fricke GM, Byrum JR, Sotiris JO, Cannon JL, Moses ME. Quantitative measurement of naïve T cell association with dendritic cells, FRCs, and blood vessels in lymph nodes. Frontiers in Immunology 9, 2018. doi: 10.3389/fimmu.2018.01571.
75. 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. Physiological Reports 9, 2021. doi: 10.14814/phy2.15147.
76. Thomas DT, Erdman KA, Burke LM. Nutrition and Athletic Performance. Medicine and Science in Sports and Exercise 48: 543–568, 2016. doi: 10.1249/MSS.0000000000000852.
77. Toth MJ, Goran MI, Ades PA, Howard DB, Poehlman ET. Examination of data normalization procedures for expressing peak VO, data [Online]. www.physiology.org/journal/jappl.
78. Tuan TC, Hsu TG, Fong MC, Hsu CF, Tsai KKC, Lee CY, Kong CW. Deleterious effects of short-term, high-intensity exercise on immune function: Evidence from leucocyte mitochondrial alterations and apoptosis. British Journal of Sports Medicine 42: 11–15, 2008. doi: 10.1136/bjsm.2006.029314.
79. Turner JE, Wadley AJ, Aldred S, Fisher JP, Bosch JA, Campbell JP. Intensive Exercise Does Not Preferentially Mobilize Skin-Homing T Cells and NK Cells. Medicine and Science in Sports and Exercise 48: 1285–1293, 2016. doi: 10.1249/MSS.0000000000000914.
80. van der Windt GJW, Everts B, Chang CH, Curtis JD, Freitas TC, Amiel E, Pearce EJ, Pearce EL. Mitochondrial Respiratory Capacity Is a Critical Regulator of CD8 + T Cell Memory Development. Immunity 36: 68–78, 2012. doi: 10.1016/j.immuni.2011.12.007.
81. Voss K, Hong HS, Bader JE, Sugiura A, Lyssiotis CA, Rathmell JC. A guide to interrogating immunometabolism. Nature Reviews Immunology 21: 637–652, 2021. doi: 10.1038/s41577-021-00529-8.
82. Wadley AJ, Cullen T, Vautrinot J, Keane G, Bishop NC, Coles SJ. High intensity interval exercise increases the frequency of peripheral PD-1+ $CD8^+$ central memory T-cells and soluble PD-L1 in humans. Brain, Behavior, & Immunity - Health 3: 100049–100049, 2020. doi: 10.1016/j.bbih.2020.100049.
83. Withnall E, Hazeldine J, Llibre A, Duggal NA, Lord JM, Sardeli AV. Physical activity modifies the metabolic profile of $CD4^+$ and $CD8^+$ T cell subtypes at rest and upon activation in older adults. .
84. Yaqoob P, And D, Calder PC. Glutamine Requirement of Proliferating T Lymphocytes. OElsevier Science Inc, 1997.
85. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. The Journal of the American College of Dentists 81: 14–18, 2014.

Instructions for authors of EIR

EIR usually solicits papers from authors with acknowledged expertise in the field to be covered. Unsolicited papers will be considered and can also be accepted. All papers are subject to a peer review process. Usually the manuscripts will fit into one of two major categories: I) A review which thoroughly covers the area indicated in the heading and includes structuring and critical discussion of existing knowledge and, if possible, the ideas of the authors about potential practical consequences and future developments. Mere mentioning and listing of existing literature is not considered to be a good review. The review can be long, if necessary, or short, if the field covered by the heading is relatively new or very focussed. II) a paper showing original data accompanied by an extended, review-type discussion. The general format of the review is somewhat flexible. A review must however have an abstract, an introduction and a conclusion around the main sections. Reviews with three or more sections should list the headings of the sections in form of a bullet point table at the end of the introduction. Longer sections should also give a short interim summary at their end. If substantial amounts of the authors‘ own new data are to be shown, a section on methods and on results must be included. Data will only be accepted, if methods are stated clearly and appropriate statistical evaluation of results is given. Other types of papers, eg true meta-analyses of a circumscribed sector of literature or papers focussing on new ideas or hypotheses may also be considered. Interested authors, please contact the editorial board. For reference style use the one as applied by J. Appl. Physiol., with references listed in alphabetical order. In text use ref. numbers in brackets. When giving more than 1 reference in one bracket, use numerical order. The maximum number of references is normally 150. A short running head should appear after the title, followed by the authors and their respective affiliations. The full address of correspondence should include an e-mail address of the correspondent author. Up to five key words should be added after the abstract. Please use e-mail for all communications including manuscript submission (word or pdf-file) if possible and paste „EIR” in the subject field of your mailing program. Generally, submission deadline for the next issue of EIR is September, 31th.

Exercise Immunology Review is an open access journal organized at cost. There is only an acceptance fee of 480 Euro/US$ to cover the production costs.

Send manuscripts to: Subject: EIR
Prof. Dr. Karsten Krüger
Department of Exercise Physiology and Sports Therapy
Institute of Sports Science, University of Giessen, Germany
Kugelberg 62 | 35394 Giessen
Editor-in-Chief: Prof. Dr. Karsten Krüger

To subscribe or renew subscription go to https://exerciseimmunology.com.