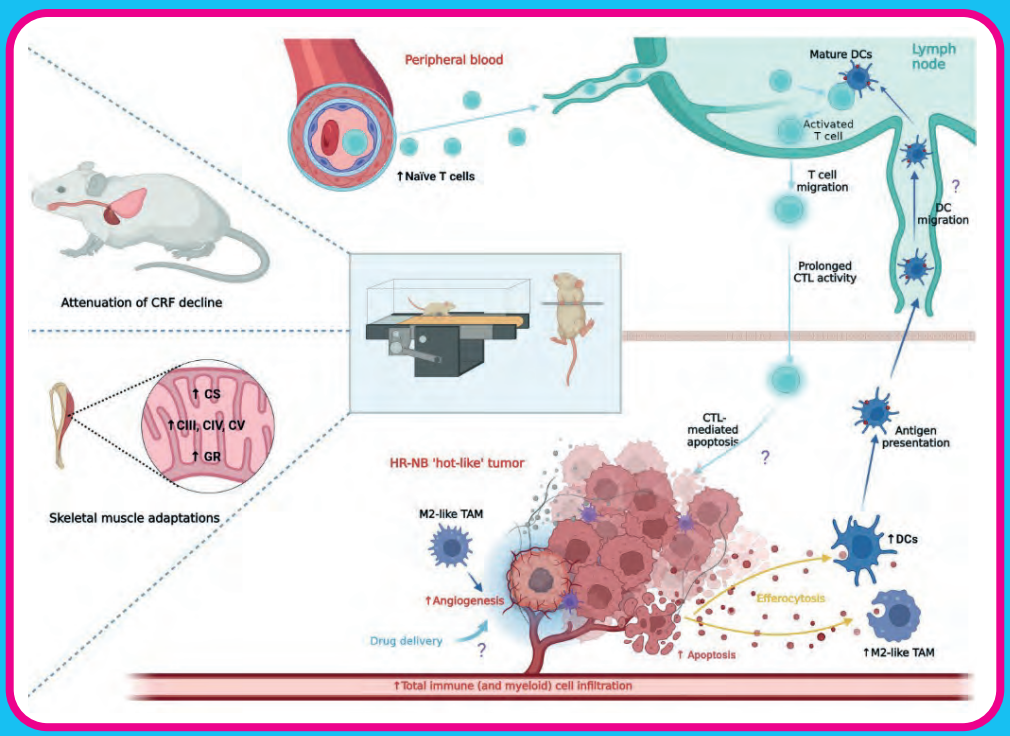
EXERCISE IMMUNOLOGY REVIEW



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EXERCISE IMMUNOLOGY REVIEW

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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.

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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

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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

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Respiratory viral infections – impact on sport and exercise medicine

Raakel Luoto[1], Matti Waris[2], Maarit Valtonen[3], Olli Ruuskanen[1*]

[1] Department of Pediatrics and Adolescent Medicine, Turku University Hospital and University of Turku, PL 52, 20521 Turku, Finland, rajopi@utu.fi, olli.ruuskanen@tyks.fi
[2] Institute of Biomedicine, University of Turku and Department of Clinical Virology, Turku University Hospital, Kiinamyllynkatu 13, 20520 Turku, Finland, mwaris@utu.fi
[3] Finnish Institute of High Performance, KIHU, Rautpohjankatu 6, 40700 Jyväskylä, Finland, maarit.valtonen@kihu.fi

ABSTRACT

Respiratory viruses are the most frequent causative agents of disease in humans and thus also in elite athletes. The COVID-19 pandemic has recently emphasized the entire spectrum of respiratory tract infections worldwide. Understanding the basic elements of respiratory viral infections is a fundamental requirement from the perspective of etiological diagnostics, treatment, and prevention strategy planning, as well as resource allocation.

Keywords: respiratory virus, acute respiratory infection, common cold, exercise, sport, athletes

**Correspondence to:*
Prof Olli Ruuskanen, MD, PhD; Department of Pediatrics and Adolescent Medicine, Turku University Hospital, PL 52, 20521 Turku tel: +358 40 7486250, email: olli.ruuskanen@tyks.fi

Abbreviations: ARI – acute respiratory infection; Ct value – cycle threshold value; EBV – Epstein-Barr virus; HEPA filter– high efficiency particular air filter; IOC – International Olympic Committee; NAAT – nucleic acid amplification test; POCT – point of care test; PCR – polymerase chain reaction; RT - reverse transcription

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ORCIDs
Raakel Luoto: 0000-0001-5752-7047
Matti Waris: 0000-0002-6747-0889
Maarit Valtonen: 0000-0001-8883-2255
Olli Ruuskanen: 0000-0002-4354-1187

INTRODUCTION

Respiratory viruses are the most frequent causative agents of disease in humans and the most common reason to seek medical care. Globally, acute respiratory infections (ARIs) represent an enormous social and disease burden with 17 billion cases and an estimated 120 million disability-adjusted life years annually (41, 162). In a 9-month follow-up study with 15112 participants, febrile ARIs led to an overall reduction in exercise equivalent to 15% of the active US population becoming completely immobilized for 1 day (106). The importance of viral respiratory tract infections has been emphasized recently because of the emergence of the coronavirus disease COVID-19 leading for 2020 and 2021 to an estimated half a billion cases and 15–18 million excess deaths (20, 114). Diagnostics for respiratory virus detection has evolved from viral culture tests to the current standard nucleic acid amplification tests (NAATs), to multiplex point-of-care tests (POCT), and now at-home tests (8, 18, 137). The clinical impact of viral diagnostics has been shown in numerous studies (179). The main value of viral testing has been to differentiate between viral and bacterial infections, to reduce unnecessary antibiotic use and to detect influenza and COVID-19 for possible antiviral treatment (96, 144, 147, 181). However, what is not well understood is the relative contribution of the various modes of transmission of different viruses (79, 91, 105, 174).

In this review we provide a clinically relevant overview of the epidemiology, clinical manifestations, transmission, virological diagnosis, and prevention of acute respiratory viral infections. At the end of relevant section, we provide a brief conclusion on the impact of the topic on sport and exercise medicine.

RESPIRATORY VIRUSES

There are ten different respiratory virus groups circulating constantly in the community: rhinoviruses, human coronaviruses (seasonal 229E, OC43, HKU1, NL63, and the emerged SARS-CoV-2), respiratory syncytial viruses, influenza viruses (A, B and C), adenoviruses, enteroviruses, parainfluenza viruses (types 1–4), human metapneumoviruses, human bocaviruses, and Epstein-Barr virus (EBV). They are all recognized as being adapted to efficient person-to-person transmission (12, 18, 58, 117, 165). There are hundreds of sub- and serotypes of these viruses but the clinical significance of more detailed diagnostic recognition of viruses is low. Rhinoviruses and seasonal coronaviruses are the most common viruses constituting 60–80% of detected viruses from patients with acute respiratory illness (1, 13, 118).

Viruses are formed by a single- or double stranded nucleic acid (DNA or RNA) which is surrounded by a protein capsid. RNA viruses include rhinoviruses, coronaviruses, influenza viruses, parainfluenza viruses, respiratory syncytial viruses, metapneumoviruses and enteroviruses. Adenoviruses, bocaviruses and EBV are DNA viruses. Most human respiratory viruses have an outer envelope composed of lipids and proteins. The most common respiratory virus, rhinovirus is small (diameter 30 nm) non-enveloped virus, as well as adenoviruses, bocaviruses and enteroviruses. There are 3 rhinovirus species, A, B and C. The total number of rhinovirus types is 169 (65). The other common respiratory virus, human coronavirus (Figure 1) is enveloped virus with a diameter of 80–120 nm and around 30 kilobases long genomes, the largest among the known RNA viruses (36).

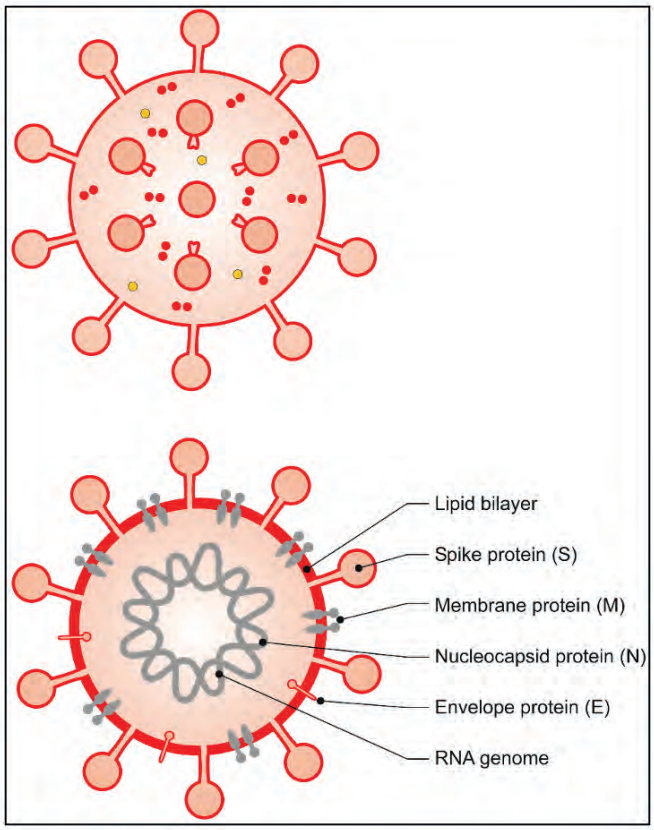

Figure 1. Schematic depiction of the coronavirus structure.

DISEASE BURDEN

In family studies conducted over 50 years ago, the annual frequency of ARI in young adults was 2.3–4.8 episodes (110). In a recent study in the USA (13), 26 households with 105 individuals were followed for one year with weekly symptom diaries and the collection of nasal swabs for viral diagnostics. In 26 of the participants aged between 18–39 years, the mean rate of ARI episodes was 4.6 and a virus was detected in 6.3 episodes per person per year, i.e. approximately 30% of the infections were asymptomatic. Children younger than 5 years of age were virus positive half of the year (50% of the weeks of the follow-up) and young children in the household were considered to be the major risk factor for virus transmission. In an internet-based surveillance 125 participants between 15–34 years of age reported an average of 3.7 ARIs during a 1-year period (3). The annual mean number was recently estimated to be globally 2.25 episodes (71). In young adults (18–49 years of age), a lower incidence of 0.46 was recently reported in a 5-year text message study (158). It is worth noting, that respiratory viruses induce recurrent infections because they do not elicit long-term protective immunity (32, 138).

Impact on sport and exercise medicine. An upper respiratory infection, presenting as a common cold before or during a major competition may ruin an athlete's long-term goal and, in addition, cause marked financial losses in professional sport. Some athletes may be exceptionally prone to respiratory viral infections and suffer prolonged symptoms (22, 56, 159). Genetic susceptibility to respiratory viral infections is, however, not well understood (89, 99). Heavy exercise and psychological stress are traditionally believed to increase the risk of infection, but clinical and virological evidence is lacking (33,45,144).

A recent review and meta-analysis of the International Olympic Committee (IOC) Consensus group reported an annual ARI incidence of 1.7 in athletes (26, 154). The incidence is lower or comparable with that of the normal population (3, 13, 110). The real occurrence of respiratory viral infections in athletes in different disciplines is rarely studied in long-term controlled studies with viral diagnostics (52, 144).

EPIDEMIOLOGY AND SEASONALITY

Respiratory viruses typically display three different patterns of occurrence: endemic, endemic with seasonal peaks, and epidemic (113). Adenoviruses are typically endemic as their prevalence is static, not exhibiting any significant rise or not fall. Many respiratory viruses are epidemic, displaying substantial seasonal variation, i.e. the prevalence occurs at regular up and down intervals (Figure 2). The most common respiratory viruses, rhinoviruses can be detected throughout the year, but distinct peaks occur in early autumn and in late spring. Four seasonal coronaviruses cause outbreaks in the winter months (85, 98). Influenza epidemics have been well known for a long time, the Spanish influenza pandemic of 1918–1919 as "the mother of all pandemics" (163). In temperate climates, influenza epidemics occur yearly with a peak prevalence in winter months in the Northern Hemisphere

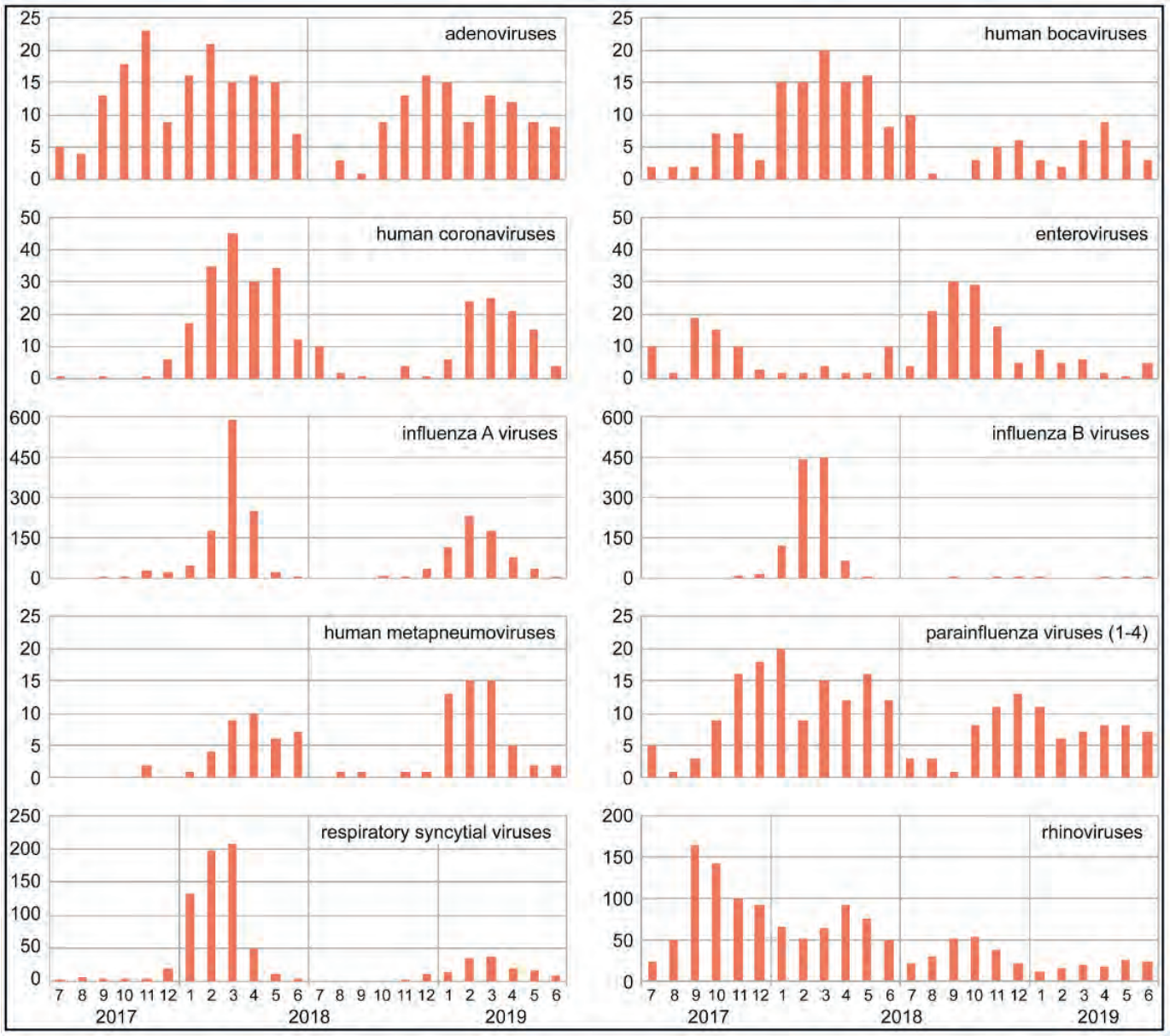

Figure 2. Seasonality of respiratory viruses. Monthly numbers of detections during two respiratory virus seasons (from July 2017 to July 2019) in Turku, Finland. Magnitudes are not comparable between viruses due to differences in the numbers of tests performed. Data from the Turku University Hospital Laboratories, Department of Clinical Microbiology.

and in summer months in the Southern Hemisphere. Globally, annual epidemics of influenza cause 3–5 million cases of severe illnesses and about 500 000 deaths, mostly among the elderly (63). Respiratory syncytial virus, the leading cause of lower respiratory tract infections in children, induces regular severe epidemics. In most countries these infections occur annually during the winter months. Epidemics usually last 3–4 months (123). It must be stressed that during influenza or respiratory syncytial virus epidemics many other respiratory viruses like seasonal coronaviruses also circulate in the community and cause respiratory infections (98, 117). Whether SARS-CoV-2 establishes a seasonal pattern similar to other coronaviruses remains to be seen. Overall, viral lows tend to constantly occur during the summer months. In tropical areas, many respiratory viral infections are endemic, and some occur during the rainy season (39, 58). In the subtropics, e.g. China and Hong Kong, influenza A may occur in two yearly epidemic (183).

The reasons for the seasonality of respiratory viruses are not well understood. Three mechanisms have been proposed: 1. Environmental parameters. At low temperature and low humidity respiratory viruses in aerosols are more stable and transmissible. 2. Human behavior. Indoor activities, crowding, number of close contacts, opening of schools and traveling all increase person-to-person contacts and transmission of viruses. 3. Host defense. Antiviral immunity may have seasonal changes. The dry air in the wintertime impairs the function of cilia in the airway epithelium. Seasonal molecular changes in immunity have also been recorded. Importantly, the innate response to rhinovirus, the most common causative agent of the common cold, is lower at low temperatures (79, 91, 92, 113,121, 174).

The human-environmental interaction affecting the seasonality of respiratory viruses is complex. No single mechanism seems to be a major factor. In some countries like Finland, large respiratory syncytial virus epidemics occur only every other year in 2-year cycles suggesting that environmental factors may be less important (175). Recently, a great number of studies have shown that COVID-19

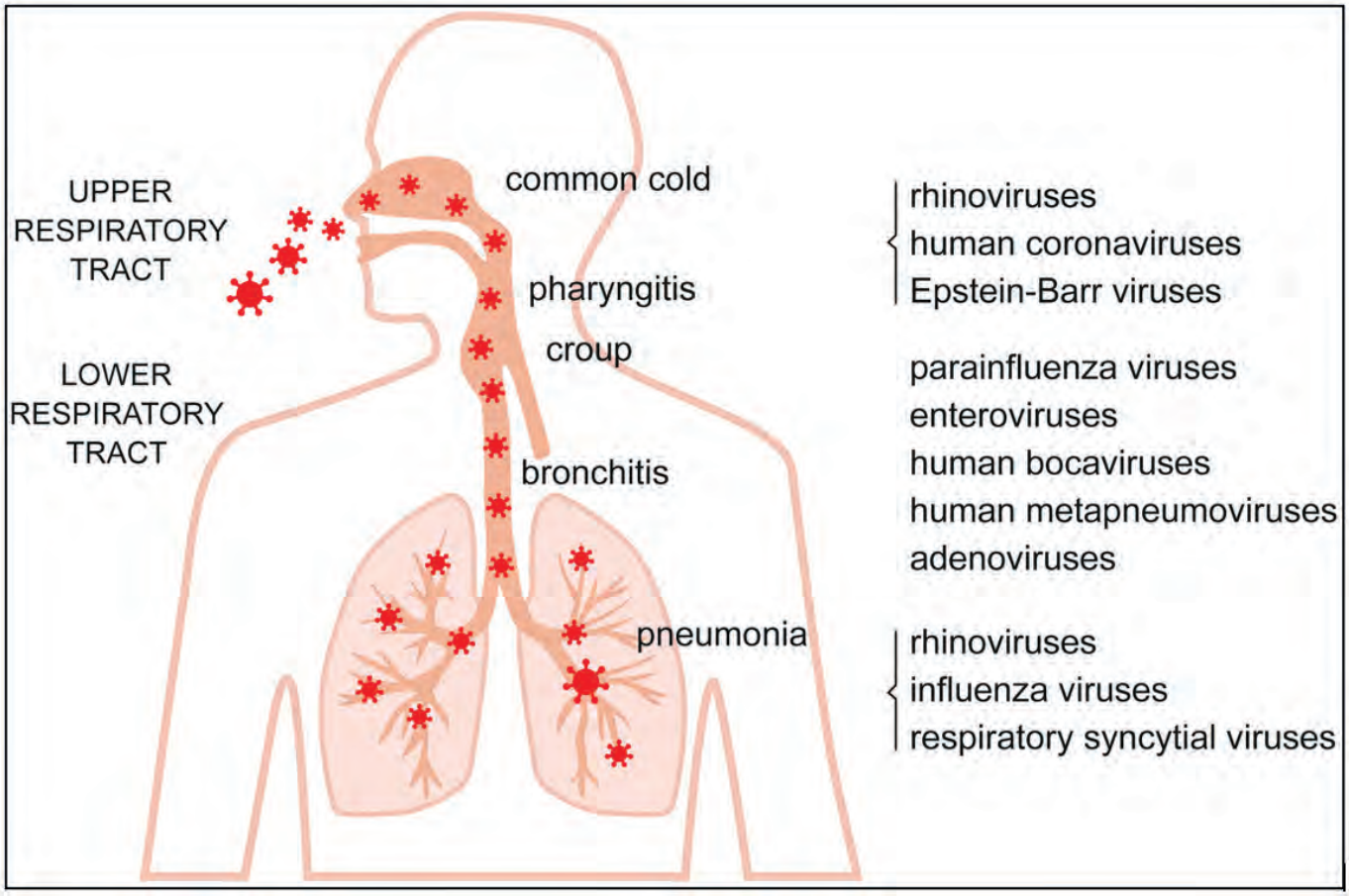

Figure 3. Human respiratory tract and the most common clinical presentations associated with different respiratory virus groups causing respiratory tract infections in humans. Classification of upper and lower respiratory tract infections is nonspecific because all respiratory viruses can cause both upper and lower respiratory tract infections.

prevention procedures, wearing masks, maintaining physical distancing and sanitizing, nearly eliminated the occurrence of other respiratory viral infections, most effectively influenza and respiratory syncytial virus epidemics (48, 124, 140, 182). Likewise, consistent with the reduction in preventive measures, the return of non-SARS-CoV-2 respiratory viruses has been observed (17, 51). These observations clearly support the important role of human behavior in the seasonality of respiratory viral infections. For example, the school calendar predictably drives seasonal variation in the prevalence of the common cold (rhinovirus) (34).

Impact on sport and exercise medicine. It is important to realize that seasonality affects much on athletes' susceptibility to respiratory viral infections. During winter games the athletes have a significantly increased risk for viral ARIs probably due to the marked environmental viral pressure in the community (27, 168, 170). Furthermore, high risk crowded indoor gatherings with poor ventilation are more common during wintertime (57).

CLINICAL MANIFESTATIONS

Symptomatic infections

The symptoms of an ARI arise after an incubation period (the time between being infected with a virus and the onset of symptoms) of 1–5 days for most viruses (90). The common cold ("a cold") is the most common self-limited clinical manifestation of all respiratory viral infections. The clinical features include a sore throat, sneezing, a runny nose, nasal stuffiness, and a cough. Fever is not a common manifestation in adults (55). In addition to the common cold, the clinical presentations of ARI in adults include pharyngitis, tonsillitis, bronchitis, and pneumonia. Classification into upper and lower respiratory tract infections is partly artificial because symptoms like a cough commonly occur in both infections and all respiratory viruses can cause both upper and lower respiratory tract infections (Figure 3) (133, 152). The significant overlap in clinical symptoms that exist with the different viruses causing respiratory illnesses and the viral etiology of these illnesses means that they are unlikely to be reliably distinguished by their clinical features alone (101). Generally, the severity of the symptoms increases rapidly, peaks within 2–3 days and decreases soon after (31, 55, 180). The mean duration of the common cold is 7–10 days and in practice all patients make a full clinical recovery within 21 days after the onset of the symptoms (1, 133, 134, 143). In one study the mean duration of the cough was found to be 11 days (133). Clinically, the illness is usually mild (symptoms do not interfere with daily activities) or moderate (symptoms interfere with daily activities). Severe respiratory viral infections (symptoms prevent daily activities, require medical consultation or hospitalization) are rare and usually only occur in high-risk patients (4). Individuals at increased risk for disease severity include infants, senior citizens (>65 years old), obese individuals, patients with underlying respiratory conditions and those with a suppressed immune function. The duration of infectivity of different viral infections varies but are usually most infectious for 1–3 days and may continue up to 5–10 days after the onset of symptoms (55).

Asymptomatic infections

Many studies have shown that asymptomatic respiratory viral infections are much more common than has been understood earlier (67, 129). In the family study by Byington and associates, 44% of the 783 detected viral episodes were asymptomatic (13). In another family study investigating influenza transmission in families, 11% of the influenza positive individuals were asymptomatic (62). The high prevalence of asymptomatic SARS-CoV-2 positivity (up to 50% of the positive total) (136) and transmission of the virus from asymptomatic individuals have recently been addressed in several different studies (77). Some of the investigators consider respiratory viruses in asymptomatic subjects as innocent bystanders and consider the potential causal role of some viruses detected by PCR questionable (139). However, respiratory RNA viruses (with exception of coxsackieviruses) do not cause chronic asymptomatic infections and most probably meaningless carriage does not occur in healthy humans (171). To what extent an asymptomatic person can transmit the infection and how often asymptomatic infections develop into symptomatic infections is still unclear.

Clinical features of specific respiratory viruses

Although all respiratory viruses may cause upper and lower respiratory tract infections, they can also have typical clinical manifestations (Figure 3). The rhinovirus is the common cold virus causing about 50% of the cases and, in addition to upper respiratory tract infections rhinoviruses are associated with a wide range of clinical presentations. They are the most common cause of acute wheezing in children older than 2 years and acute asthma exacerbation in adults. They can cause pneumonia and induce exacerbation of chronic obstructive pulmonary disease (1, 64, 65, 117, 146).

Seasonal coronaviruses (non-SARS-CoV-2 coronaviruses) are common cold viruses (32, 85, 98). In one study coronaviruses were identified in 22% of young adult ARI patients with a cough and a chill (23). Coronaviruses may also induce severe respiratory infections in adults necessitating hospitalization and intensive care treatment (44, 173). The clinical spectrum of the SARS-CoV-2 infection varies from a mild common cold to severe pneumonia with respiratory failure and a multiple organ dysfunction syndrome. Originally, the major COVID-19 symptoms were fever, a persistent cough, fatigue, and a loss of or a change in taste or smell (46). Currently, with the dominance of the Omicron variant, the symptoms are usually mild including rhinorrhea, a sore throat, fatigue, and a headache; all these symptoms are also symptoms of all the other respiratory viruses (166). Thus, the majority of the non-hospitalized patients have an uneventful recovery. However, in one follow-up study of non-vaccinated patients at 12 months only 23% of patients (32% hospitalized) were completely free of symptoms (150). Fatigue, reduced exercise capacity, sleep difficulties and neuropsychiatric symptoms are the most common persistent symptoms after recovering from COVID-19 (109, 150).

Influenza ("flu") is characterized by the abrupt onset of fever, chills, myalgia, a headache, malaise, a sore throat, and rhinitis. A nonproductive cough may last 4–6 weeks after an acute illness (167). In one study 24% of the cases were asymptomatic or paucisymptomatic (had 1 symptom only) (62). A febrile illness with a cough or sore throat but without laboratory diagnosis of influenza is referred to as an influenza-like illness (106).

Worldwide, respiratory syncytial virus is the single most frequent cause of lower respiratory tract infections (bronchiolitis and pneumonia) in young children (47). In older children and young adults, a respiratory syncytial virus infection is a mild common cold (119). The main symptoms in healthy young adult military recruits were a sore throat (76%), sputum (73%), a cough (72%), tonsillar hypertrophy (68%), and rhinorrhea (56%) (126). In elderly people, respiratory syncytial virus infections are a significant cause of mortality, with over 6000 patients a year in the US alone (53).

The adenovirus induces symptomatic respiratory infection usually in children less than 5 years of age. Adenoviruses are also an important cause of childhood diarrhea. Infections are rare in adults but, interestingly, up to 80% of military recruits may experience an adenovirus infection occurring most commonly in training weeks 3 to 6. The clinical manifestations include fever, conjunctivitis, a sore throat, a cough, and pneumonia. An oral vaccine for adenovirus types 4 and 7 is effective but used only for the military basic training population in the US (97, 145).

Parainfluenza viruses are associated with upper and lower respiratory tract infections. They account for 75% of croup cases. Hoarseness, a barking cough, and inspiratory stridor are the classic signs (6, 141).

EBV seroconversion occurs usually during childhood. Clinically symptomatic infection may occur when primary infection is delayed. EBV is associated with approximately 8% of sore throat cases among adolescents (30). In young adults, EBV infection induces acute infectious mononucleosis with an incidence of 6–8 /1000 (30). The classic triad of symptoms includes fever, tonsillitis, and lymphadenopathy. Splenomegaly, palatal petechiae and fatigue are also common. EBV-induced mononucleosis is diagnosed by serology using a specific IgM test. Fatigue may persist for 4–6 months (14). Splenic rupture is a rare but potential complication. That is why physical activity, especially contact sport, is prohibited within the first 30 days of the illness (161).

Impact on sport and exercise medicine. In 6 etiological studies carried to date the etiology and clinical manifestations of respiratory viral infections in 178 athletes were comparable to those in young adults in the general population. Rhinoviruses, seasonal coronaviruses, influenza viruses, and respiratory syncytial viruses were the most commonly detected viruses (21, 49, 118, 156, 168, 170). The clinical manifestations were in practice only upper respiratory symptoms (the common cold) including a sore throat, a runny nose, and nasal stuffiness. The duration of ARI was 5–7 days, which agrees with the mean duration of 7 days reported by the IOC meta-analysis (153, 154).

As is commonly known, elite athletes often train and compete while suffering from mild upper respiratory symptoms. The effect of a mild viral infection on performance and the health risks of physical exercise during infection

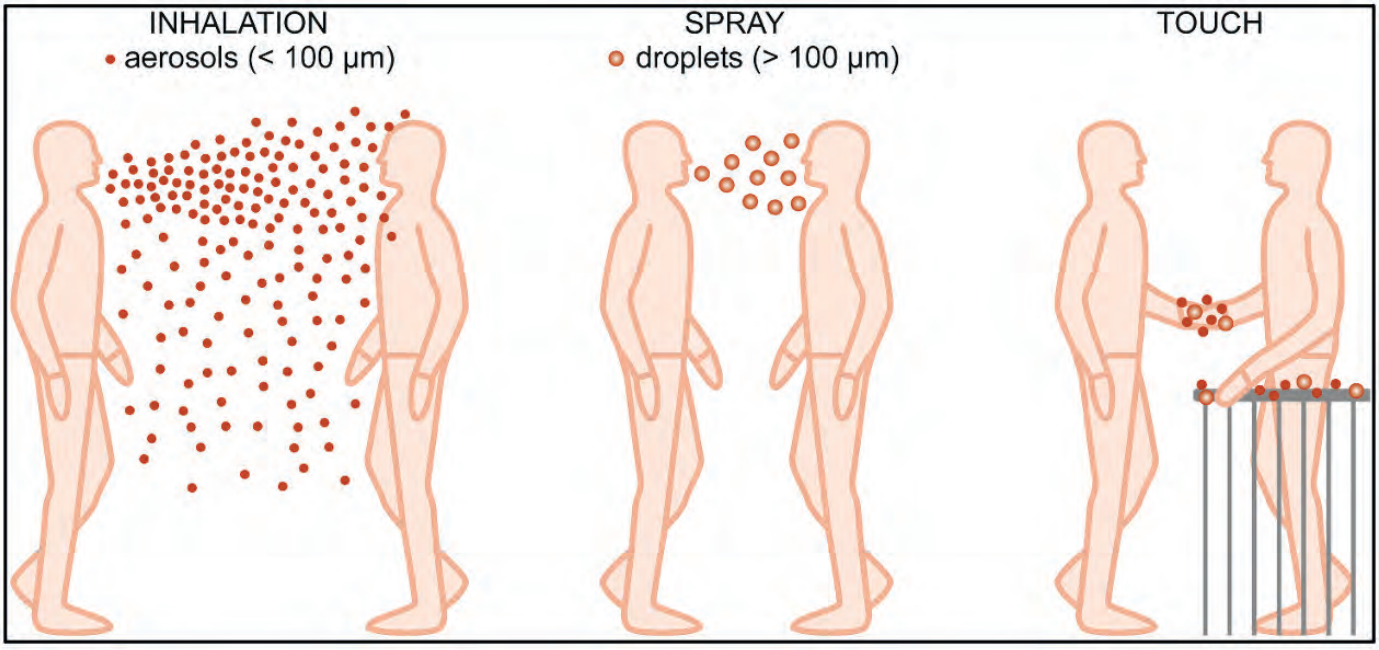

Figure 4. The transmission mechanisms of respiratory viruses: inhalation, spray, and touch.

are poorly understood (75). In one study on international swimmers, mild previous illness had trivial to small effects on competitive performances (135). The risk of myocarditis exists. However, of a total of 7988 athletes who recovered from COVID-19 the rate of myocarditis was 1% (108). In the absence of evidence-based return-to-sport protocols, clinicians rely on self-monitoring and the response to a gradually increased exercise regimen (153). No activity restriction is needed if an athlete has no cardiac symptoms such as chest pain, palpitation, or dyspnea (52, 83). The common-sense approach has not presented significant evidence of any harm to the athletes.

VIRAL-BACTERIAL CO-INFECTIONS

It has been well known for many years that a respiratory viral infection may pave the way for a secondary bacterial infection (acute otitis media, sinusitis, pneumonia) (35, 107, 112). Rhinovirus infection increases the acquisition and transmission of *Streptococcus pneumoniae* (74). We have shown that rhinovirus circulation in the community had an association with invasive pneumococcal disease in children (128). There is also evidence that mixed viral-bacterial infections induce a more severe clinical disease than individual bacterial or viral infections (100, 133, 143). As many as 20% to 60% of young children with respiratory viral infections develop bacterial acute otitis media (142). In young healthy adults, bacterial complications are rare. Bacterial sinusitis develops in only 1–2% of cases (73). In hospitalized patients with COVID-19 bacterial co-infections have been detected in 5–10% of the cases (111). It is of interest that pneumococcal vaccination confers moderate protection against respiratory viruses associated with lower respiratory tract infections in children and adults. Pneumococcal vaccination diminishes pneumococcal carriage which reduces the likelihood of acquiring respiratory viruses (94).

Impact on sport and exercise medicine. Bacterial co-infections of respiratory viral infections in athletes are uncommon and antibiotic treatment of ARI is rarely indicated (168, 169, 170).

TRANSMISSION

Recently, a simplification to 3 major routes of transmission has been proposed (104) (Figure 4):

1. Inhalation. Viral transmission occurs by direct inhalation of airborne particles (aerosols) containing viruses and viruses are deposited at various sites in the respiratory tract, depending on the size of the aerosol (105). Small aerosols are deposited straight onto the alveoli in the lungs. Aerosols are now defined as particles less than 100 μm in diameter. Aerosols are produced during breathing. It is of importance that speaking increases aerosol production by 35-fold, exercise, and heavy breathing by 60-fold, and coughing by 400-fold (79). Virus-laden aerosols can remain infectious in indoor air for hours and can be carried beyond 2 m from the infectious person and everywhere in the room without any safe distance (91, 174).

2. Spray. Particles with more than 100 μm in diameter are called droplets. Virus-laden droplets transmit the infection only when susceptible individuals are within 0.2 m of a talking infectious person. Sneezing and coughing propel the droplets further. Droplets settle down onto the ground within 2 m of the source person within a few seconds. Droplets transmit the infection through direct spray from the infector to the mucosa of the nose, eyes and mouth of the susceptible person (104).

3. Touch. Transmission is introduced by hands. Susceptible individuals shake hands with an infected individual whose hands are contaminated with a virus and then introduce the virus to his mucous membranes. The other possibility is to pick up the virus by hand from highly touched and contaminated surfaces (e.g. door handles, desks, elevator buttons, mobile phones). Respiratory viruses may remain infectious on surfaces from a few hours to several days (81, 82, 177). However, the

spread of respiratory viruses through touch in real-life is poorly documented

Transmission risk factors

Time spent in a crowded and poorly ventilated indoor space is the key determinant in the transmission of respiratory viral infections (105). Timing after the onset of infection is also critical, the highest frequency of transmission to contacts being most likely at the peak viral load and symptom severity (usually 1–3 days after the onset of symptoms) (40). Importantly, the risk of transmission is negligible outdoors (10). Close contact requires a 2-way face-to-face conversation of ≥3 words, close proximity, physical contact, or some combination of those criteria (57). Close contact has occurred when a person is less than 2 m away from the infector for 15 minutes or more over a 24-hour period (16). Potential high-risk transmission settings include a household, shared rooms, a concert, or a sporting event and public transportation vehicles like an aircraft, a bus, or a train. The household is the most common potential high-risk transmission setting. In our own study, we detected rhinovirus infections in 50% of the adults in families with a rhinovirus-positive index child (130). Secondary attack rates in families with a SARS-CoV-2 positive case vary from 22% to 43% (102). In a train, passengers seated next to a person with symptomatic SARS-CoV-2 were 10 times more likely to catch an infection compared to those sitting 3 seats away (60). The risk of influenza transmission on an aircraft is higher when a person is seated within 2 rows of an infector (88). On the other hand, on a 2-hour bus trip, 23 of 68 passengers were infected with COVID-19, but their seating was not significantly associated with the proximity to the index case (151).

Increasing evidence suggests that airborne transmission of numerous respiratory viruses is more prevalent than previously recognized (174). Transmissibility characteristics differ between respiratory viruses and one transmission route may dominate, for example, aerosols in COVID-19 (91, 105, 174). Studies carried out 15–25 years ago suggested that the major route of rhinovirus transmission is from the hands of an infected person to an intermediary surface or directly to the hands of the susceptible person. Recently, rhinoviruses and seasonal coronaviruses have been identified in exhaled breath and thus respiratory aerosols are now considered an important transmission route of these viruses (91, 92). Influenza transmission occurs primarily via spray and touch routes (7). Numerous studies have shown that inhalation of virus-laden aerosols is the major route of SARS-CoV-2 transmission and touch transmission is far less efficient (78, 91, 104, 105, 174).

Impact on sport and exercise medicine. It is well known that COVID-19 spread readily in sport teams like soccer and ice hockey (84, 157). Understanding the factors that shape susceptibility and transmission of viral ARIs in athletes is of fundamental importance to minimize the risk of infections. Behavioral factors may be more important than earlier understood to eliminating the risk of respiratory infection. For athletes, the frequent use of public and team transportation, human crowding, shared housing during the training camps and competitions, full-contact sports, heavy breathing and shouting by the infectees during the game and in the locker room may be important factors increasing airborne transmission. Interestingly, vigorous exercise generates smaller particles than speaking and aerosol particle emission may increase 172-fold during maximal exercise (116, 125).

DIAGNOSTICS

There are 4 major methods for the detection of respiratory viral infections: virus culture, antigen detection tests, nucleic acid amplification tests (NAATs, e.g. polymerase chain reaction (PCR) tests) and serology tests. The timeline of symptoms and diagnostic tests is shown in Figure 5. No test is absolutely accurate, meaning no test can be considered the gold standard (127, 132). It is noteworthy now that nasal mucus samples can be self-collected at home and mailed to a laboratory (149, 176).

Virus culture

In vitro cell culture for virus detection was developed in the 1940s. Different viruses induce their own particular changes in the susceptible cell lines. For many years, viral culture was considered the mainstay of viral diagnostics (61). However, viral culture requires technical expertise, is slow, time-consuming, and most importantly it has low sensitivity. In our study of 293 children with an acute respiratory infection, a viral culture detected the etiology in only 38% of the cases compared to 88% with all the 4 major methods, virus culture, antigen detection, PCR, and serology tests (68). Today, viral cultures are used in research laboratories but seldom for viral diagnostics (72).

Viral antigen detection

Protein antigens of viruses can be rapidly detected by visualizing (originally in infected cells by immunofluorescence) a specific antigen-antibody reaction. Today, a lateral flow immunoassay is the most common detection method (137). In this assay, a stationary line in a nitrocellulose paper strip is coated with an antibody against the virus. When the mucus sample containing the virus is placed on the well at the end of the strip, it becomes mixed with a secondary antibody conjugated with color particles as the liquid moves through the strip. The antigen-antibody complexes formed react with the stationary antibody and a visible line of color appears if the viral antigen is present in the sample. Several at-home rapid diagnostic tests have also recently emerged for influenza and the respiratory syncytial virus. Antigen tests are easy to use, they have a 15–20 minutes turnaround time, and they are inexpensive. However, it must be remembered that except for SARS-CoV-2, antigen tests for respiratory viruses are not recommended for adults due to the low sensitivity that is derived from low viral loads (9, 42, 80).

Nucleic acid amplifications tests

NAATs were developed during the 1990s and have become the workhorse of virus diagnostics. Briefly, DNA and/or RNA are purified from the sample, added to a mixture of nucleotides, oligonucleotide primers, probes, and a polymerase enzyme. As the target is often an RNA virus, PCR is preceded with a reverse transcription (RT) step converting RNA to a complementary DNA. RT-PCR can be performed as a single run by including the RT enzyme into the reaction. During PCR, progeny strands are synthetized by the action of the DNA polymerase. The target sequence is amplified in 35–45

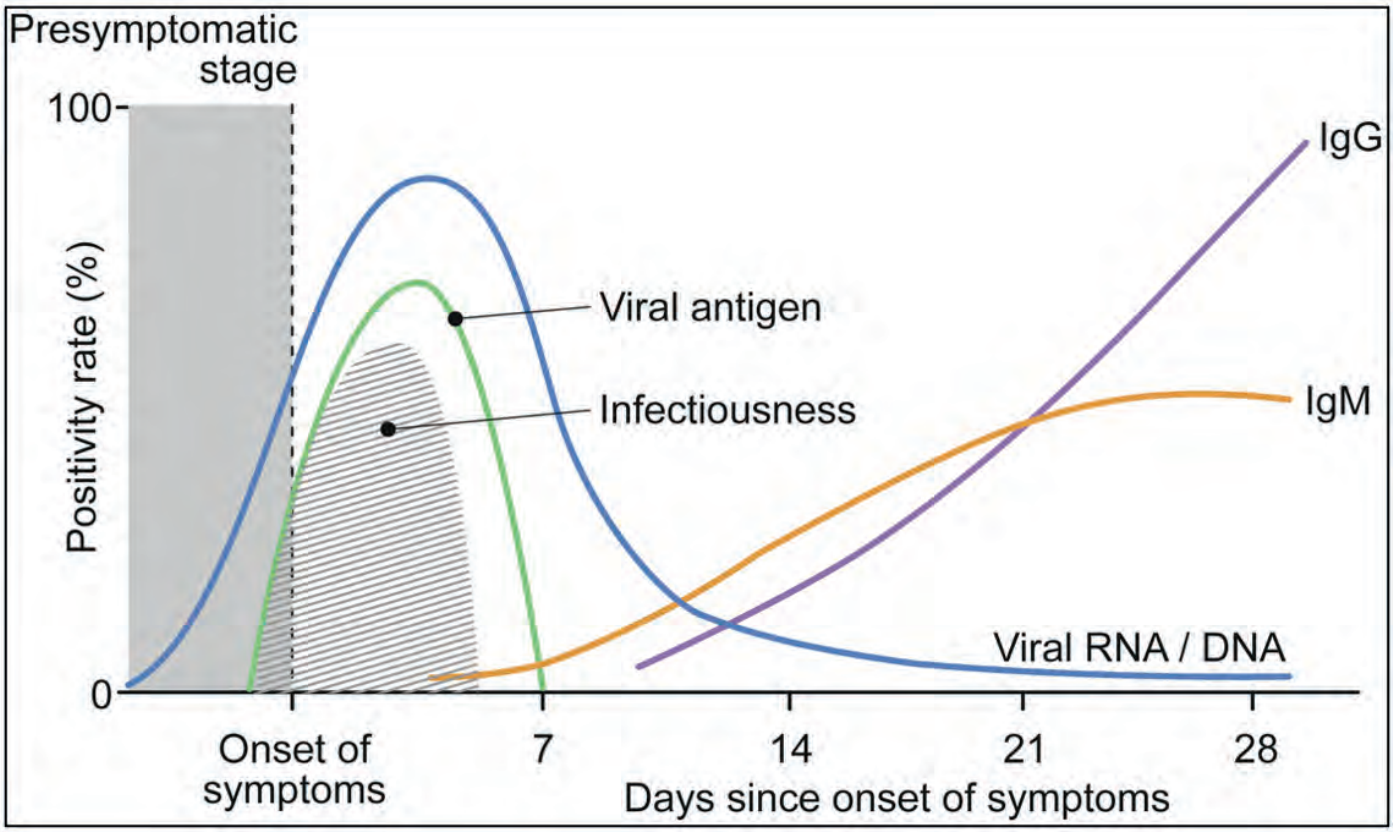

Figure 5. Generalized timelines for infectiousness, host immune response and optimal use of different diagnostic tests (nucleic acid amplification tests, antigen tests and serology) for respiratory viral infection.

temperature cycles. A fluorescent probe is used to demonstrate the reaction. PCR is highly sensitive and specific at detecting RNA or DNA of respiratory viruses. However, the sensitivity does not reach 100%. For example, PCR was able to detect only 80% of symptomatic influenza infections (86). This low sensitivity may be due to the nonoptimal timing of the sample, specimen quality and type and performance of the actual assay (86, 132). It is also possible, that there may be uncharacterized respiratory viruses awaiting discovery.

Virus sequencing is used in infectious disease epidemiology to decipher the genomic material of the virus for variant identification and to track the spread of infection. Sequencing has been especially important in the detection of various SARS-CoV-2 variants during the global spread of infections.

Multiplex PCR tests

Over the past decade, PCR tests have been multiplexed and nowadays it is possible to screen up to 16–20 respiratory viruses concurrently from a single mucus sample (155). The viral recovery rate with multiplex PCR is usually 40–60% (70, 158). The sensitivity of multiplex PCR tests is not as high as that of PCR tests for detecting a single virus. It should also be emphasized that in contrast to a positive virus culture a PCR test is not able to distinguish whether an infectious virus is present. Respiratory viruses do not induce chronic infections (171). PCR tests turn negative after acute infections usually within 1–2 weeks (viral shedding time) (Figure 5) (129). SARS-CoV-2 and bocavirus PCRs may stay positive months after an acute infection (66, 127). With the development of NAATs, it was found that in adults more than 1 virus can be detected in 5–10% of the samples. The clinical significance of viral co-infections is unclear (12, 120). In COVID-19, viral co-infections are detected in 5–10% of the cases. Co-infection with influenza was associated with an increased severity of infection (160).

The availability of the newly developed NAAT assays that can be used as POCT is increasing (8, 19). The FilmArray Respiratory Panel (BioFire Diagnostics, Salt Lake City, Utah) approved by the Food and Drug Administration is a popular test that in 45 minutes can detect 22 pathogens from a nasopharyngeal swab (61). We used FilmArray during a major winter sport event and detected respiratory viral infections on site in 88% of the 16 symptomatic cases in Team Finland. Six different viral infections were identified, all occurring at the same time in the Team (168).

Viral load

For most viruses, the current standard PCR assay for detection facilitates the estimation of the viral load in the sample. The number of cycles of the reaction at which an amplicon produces a detectable signal is the cycle threshold (Ct) value. The Ct value is inversely proportional to the amount of virus in the sample. The clinical use of Ct values expanded during the COVID-19 pandemic. It is well established that SARS-CoV-2 Ct values of <25 generally demonstrate an infectious virus (11, 69). With respect to the other respiratory viruses, the available studies have provided conflicting results about the correlation between the viral load and either infectiousness or a correlation to the severity of the illness (5, 37).

Serology

Serology tests detect specific IgM and IgG host antibody responses to an infectious virus (Figure 5). Specific IgM is

of low value in respiratory virus diagnostics but will identify recent infection. A significant increase (2–4 fold) in the amount of specific IgG antibodies between acute phase and convalescent samples confirms the viral infection. Serology clearly enhances the diagnosis of respiratory viral infections, but serology is not used in everyday clinical practice because it necessitates an acute and convalescent serum sample taken 2–4 weeks apart.

Impact on sport and exercise medicine. Respiratory virus testing in athletes needs to be in a readily available format and much more widely utilized. Multiplex PCR tests are available as POCTs and permit diagnostics to be conducted at the site of the training camps and competitions (118, 168, 170).

Clinical value of virus diagnostics

Testing to treat is the optimal goal of virus diagnostics. Unfortunately, except for influenza and COVID-19, knowledge of the infecting viral agent does not usually alter the treatment; this is due to the fact, that clinically useful antiviral agents do not exist for other respiratory viruses. In hospitalized patients, the etiologic diagnosis is of importance to cohort patients, to avoid unnecessary further testing, to the use of antibiotics, and to prevent nosocomial infections (155). Additionally, laboratory confirmation of the etiology of a viral respiratory tract infection provides essential data for prevention strategies. The COVID-19 pandemic has also dramatically changed the significance of the detection of the viral etiologic agent among non-hospitalized patients.

TREATMENT

Antivirals

At present, there are no approved specific antiviral treatments for respiratory viruses other than influenza and SARS-CoV-2 viruses (24). Thus far, two first-generation oral antiviral treatments for coronaviruses have been approved for public use, molnupiravir (Lagevrio) and nirmatrelvir plus ritonavir (Paxlovid). They do not reduce the duration of symptoms but when started within 5 days of symptoms onset decrease markedly the need for hospitalization (147, 181).

Antiviral treatment with an oral neuraminidase inhibitor is recommended as soon as possible for influenza patients who are hospitalized and at risk of developing severe disease or who already have a severe, complicated, or progressive illness (15). Early (within 48 hours of illness onset) empiric antiviral treatment should also be considered for non-high-risk outpatients with suspected or confirmed influenza based on clinical judgment. The most common oral neuraminidase inhibitor for influenza is oseltamivir (Tamiflu®); while other options include inhaled zanamivir, intravenous peramivir, or oral baloxavir (15). It must be acknowledged that despite the widely accepted use of neuraminidase inhibitors, meta-analyses have shown only a modest benefit for influenza (28, 115).

Symptomatic treatment

The mainstay of the treatment for respiratory viral infection is to relieve symptoms. Effective treatment for reduction of symptom severity of viral ARI in adults is limited to over-the-counter analgesics and non-steroidal anti-inflammatory drugs intranasally or orally administered decongestants with or without antihistamines, intranasal ipratropium bromide and zinc lozenges (25, 55, 131). Antibiotics are an ineffective treatment for viral syndromes and antibiotic treatment is not indicated for adults with respiratory viral infections. Antibiotics are rather associated with potential side effects (55, 133). Antitussives have not been proven effective for cough and may even induce more harm than good (103).

Impact on sport and exercise medicine. In a recent study oseltamivir was successfully used in the treatment of influenza A outbreaks in two professional ice hockey teams (96).

PREVENTION AND CONTROL

Respiratory viral infections can be prevented by vaccination or by non-pharmacological measures to reduce transmission.

Vaccination

Vaccination is the most effective way to prevent respiratory viral infections but only two vaccines are generally available. There are three types of influenza vaccines: live attenuated, inactivated (split) whole virus, and subunit vaccine. Current seasonal influenza vaccines are multivalent with antigens from influenza A(H1N1), A(H3N2), B(Victoria), and B(Yamagata) viruses. The influenza vaccine is not an optimal vaccine because it must be given yearly and reformulated based on current circulating strains. More importantly, the effectiveness of influenza vaccines is not sufficiently adequate. During 2004–2020 the effectiveness varied from 10% to 60% (43, 122). In addition, vaccine coverage has remained far below the desired level. Influenza can also be effectively prevented by post-contact prophylaxis with oseltamivir (54).

The effectiveness (80–95%) and safety of COVID vaccines were demonstrated in a year, a record time for any vaccine (38). There is a small risk of cardiac complications after an mRNA COVID-19 vaccination, especially in young men (6 per 100 000 vaccine doses) (95). At the time of writing more than 13 billion COVID-19 vaccine doses have been delivered. Since the onset of the pandemic, new genetic variants such as Alpha, Beta, Gamma, Delta, and Omicron have emerged. Current vaccines provide little or no protection against infection with the dominant Omicron variant although they provide good protection against the severity of the disease (93). It is clear, that new vaccines (oral, nasal, inhaled) are needed.

Non-pharmacological measures

The goal of non-pharmacological measures is to minimize the interactions between susceptible and infected persons, which drive most of the viral transmission. Layered non-pharmacological preventive measures are needed to reduce the transmission of respiratory viral infections. With that in mind, it is easy to understand the importance of the following well-known measures: 1. face mask use has an effectiveness of 60–80% when both the infector and infectee use well-fitted masks (76, 79), 2. maintaining at least 1 m physical distance from other individuals, 3. avoiding nonessential crowded indoor spaces, 4. isolation of symptomatic individuals, 5.

enhancing hand hygiene by washing hands with soap for 20 seconds, 6. avoid handshaking and high-contact surfaces, 7. increasing room air ventilation e.g. opening the window and using high-efficiency particular air (HEPA) filters in closed recirculated air spaces (50), 8. active viral testing to detect community-incidence rates (59). Numerous studies have shown that these non-pharmacological preventive measures have globally controlled the spread of SARS-CoV-2 infections. Widespread use of these interventions also reduced the transmission of other respiratory viral infections including influenza virus and respiratory syncytial virus infections but less efficiently rhinovirus infections. Appropriately, it has been questioned which transmission-reducing behaviors (e.g. universal masking during the fall viral season and discontinuation of handshaking) will remain after the end of the COVID-19 pandemic to prevent the spread of other respiratory viruses. Recently, concurrent with the relaxation of government-enforced control measures, a reoccurrence of influenza viruses, respiratory syncytial virus, rhinovirus, and parainfluenza viruses has been reported (17, 51, 87). Unknown asymptomatic but infectious respiratory viral infections make prevention challenging.

Impact on sport and exercise medicine. Prevention of respiratory viral infections in athletes is a complex issue. We have no high-quality evidence of the efficacy of any single or layered intervention. The key aspects for athletes are their immunity, behavioral and environmental factors. It is generally agreed that for optimal immunity, the athlete has to balance training, nutrition and rest even though evidence of the clinical impact is lacking (172, 178). The layered mitigation procedures for COVID-19 effectively prevented the global transmission of non-SARS-CoV-2 infections in the community and subsequently in sport teams (148, 184). In the 2018 Winter Olympic Games, 45% of 44 athletes in Team Finland reported a common cold, while during the COVID-19 mitigation strategies in the 2022 Winter Olympics the corresponding percentage in 47 athletes was 6% (169). After the COVID-19 pandemic, athletes should consider using masks when traveling or when sick. Athletes who are unwell should be isolated until there is a clear improvement in symptoms (the first 1–3 days). During competitions maintaining over 2 m physical distancing is effective but may be difficult. The mitigation procedures should not induce additional psychological stress (33). Improved ventilation of indoor spaces like locker rooms is crucial. Carbon dioxide (CO2) monitoring (<800 ppm), the use of HEPA filters for removing viruses and the use of ultraviolet radiation to inactivate viruses should be considered during respiratory viral outbreaks (2, 29, 164). Awareness of viral epidemics in both the homeland and travel destination may help to regulate the need for countermeasures.

CONCLUSIONS

In this review, we have covered the basic elements of respiratory viral infections, an entirety of which every clinician who treats ARI patients should be aware of. Concerning elite athletes, ARIs during a major competition main ruin years of hard work and cause marked financial losses not only for individual athletes but also at a team level. To prevent respiratory tract viral infections, all available and evidence-based preventive, and curative modalities against ARIs among professional athletes should be immediately implemented.

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Exercise-induced effects on inflammatory markers and brain-derived neurotrophic factor in patients with knee osteoarthritis. A systematic review with meta-analysis.

Sofie Puts[1,2,3,4], Keliane Liberman[1,2], Laurence Leysen[3,4,5], Louis Forti[2], Eveline Muyldermans[4], Peter Vaes[4], Jo Nijs[3,5,6,7], David Beckwée[2,4,5,8], Ivan Bautmans[*1,2,9,10*]

[1] Gerontology department, Vrije Universiteit Brussel, Laarbeeklaan 103, BE-1090 Brussels, Belgium
[2] Frailty in Ageing (FRIA) research group, Vrije Universiteit Brussel, Laarbeeklaan 103, BE-1090 Brussels, Belgium
[3] Pain in Motion Research Group (PAIN), Vrije Universiteit Brussel, Laarbeeklaan 103, BE-1090 Brussels, Belgium
[4] Rehabilitation Research Group (RERE), Vrije Universiteit Brussel, Laarbeeklaan 103, BE-1090 Brussels, Belgium
[5] Department of Physiotherapy, Human Physiology and Anatomy, Faculty of Physical Education and Physiotherapy, Vrije Universiteit Brussel, Building F-kine, Laarbeeklaan 103, BE-1090 Brussels, Belgium
[6] Department of Physical Medicine and Physiotherapy, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, Brussels, Belgium
[7] Department of Health and Rehabilitation, Unit of Physiotherapy, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Sweden
[8] Research Group MOVANT, Department of Rehabilitation Sciences and Physiotherapy (REVAKI), University of Antwerp, Universiteitsplein 1, BE-2610 Wilrijk, Belgium
[9] Geriatrics department, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, BE-1090 Brussels, Belgium
[10] SOMT University of Physiotherapy, Softwareweg 5, 3821 BN Amersfoort, The Netherlands

ABSTRACT

Background: *In the pathogenesis of knee osteoarthritis (KOA), inflammatory mediators play an important role. However, the precise underlying mechanism by which regular exercise therapy (ET) exert effects on the immune system in KOA patients is unknown.*

Objectives: *The aim of this systematic review was to investigate the basal and acute effects of ET on inflammatory biomarkers and brain derived neurotrophic factor (BDNF) in KOA patients.*

Methods: *PubMed, Web Of Science and PEDro were systematically searched for appropriate studies. If possible, a meta-analysis was performed or an approximation of the effect size (ES) was calculated. Risk of bias was scored using the Cochrane ROB 2.0 or ROBINS-I tools.*

Results: *Twenty-one studies involving 1374 participants were included. Fifteen articles focused on basal exercise effects, four on acute effects, and two on both. Biomarker analysis (n=18) was performed in synovial fluid (n=4) or serum/plasma (n=17). A meta-analysis demonstrated that basal CRP was reduced in KOA patients 6-18 weeks weeks after ET (MD: -0.17;95%CI[-0.31;-0.03]), while IL-6 (MD: 0.21;95%CI[-0.44;0.85]), and TNF-α (MD: -0.57;95%CI[-1.47;0.32]), levels did not significantly change. Also, sTNFR1/2 did not change significantly after ET. For other biomarkers, insufficient data were available to perform a meta-analysis. Nevertheless, a low degree of evidence was found for a decrease in IL-6 (ES:-0.596 & -0.259 & -0.513), an increase in sTNFR1 (ES:2.325), a decrease in sTNFR2 (ES:-0.997) and an increase in BDNF (ES:1.412). Locally, intra-articular IL-10 (ES:9.163) increased, and IL-1β (ES:-6.199) and TNF-α decreased (ES:-2.322) after ET. An acute exercise session elicited a myokine response (ES IL-6:0.314), and an increase in BDNF (no ES-data). No inflammatory effect (ES CRP:0.052; ES TNF-α:-0.019 & 0.081) following an acute bout of training was found. However, a single bout of exercise elicited a decrease in intra-articular IL-10 (no ES-data).*

Conclusion: *ET can induce circulatory and intra-articular anti-inflammatory effects in patients with KOA. The anti-inflammatory properties have important implications for informing these patients and clinicians about the underlying effects of ET.*

INTRODUCTION

OA is a multifactorial chronic degenerative disorder, affecting the whole joint. It is mainly characterized by degeneration of articular cartilage, synovitis, and alterations in both peri-articular structures and subchondral bone (31, 36). According to the Global Burden of Disease Study of 2019, more than 527 million people are affected with OA worldwide; with the knee (364,58 million people) and hip (32,99 million people) as the most commonly affected joints (60). Clinically, OA is often associated with pain, joint stiffness, crepitus and loss of function, which can lead to disability over time. OA is one of the leading causes of global disability, accounting for 7.1% of total musculoskeletal disability burden worldwide, which is an increase of 31.4% compared to 2007 (54). This increased global burden can be explained by ageing and obesity (79).

OA has long been erroneously considered as a non-inflam-

*Correspondence to:
Ivan Bautmans – Laarbeeklaan 103, 1090 Jette, Belgium
Ivan.bautmans@vub.be

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matory arthropathy. However, it is a complex disease in which structural changes are not only caused by mechanical but also by systemic factors, such as inflammation that initiates and/or perpetuates the OA process (10, 93). Once activated by mechanical and/or systemic perturbation, joint cells (e.g. chondrocytes) and the synovial membrane in turn release inflammatory mediators into the joint cavity (73, 77). Pro-inflammatory cytokines are believed to play a pivoting role in the initiation and development of OA, specifically interleukin (IL)-1β and tumor necrosis factor (TNF)-α are key players in this process (61, 73, 77). The pleiotropic cytokine IL-6 is considered to also have a major contribution in the pathogenesis of OA (61, 73). On the other hand, there is evidence that the production of anti-inflammatory cytokines, in particular IL-4 and IL-10, can counteract the OA inflammation process and cartilage matrix degradation (61).

In addition to inflammatory cytokines, brain-derived neurotrophic factor (BDNF) is also involved in inflammatory responses (71). BDNF, a member of the neurotrophic growth factor family, contributes to neuronal development, protection and survival of neurons, and brain plasticity. About 75% of circulating BDNF origins from the brain (25). Other potential sources of BDNF include skeletal muscle, smooth muscle, platelets, endothelial cells, epithelial cells, and peripheral blood mononuclear cells (25). Additionally, BDNF can also be produced by joint cells (i.e. chondroblasts, fibroblasts) (34, 53). It is suggested that inflammation induces BDNF production and in turn, BDNF exerts pro- and/or anti-inflammatory effects in part through modulation of inflammation-related cytokines (71). Furthermore, TNF-α and IL-6 can induce BDNF secretion in human monocytes (82). To date, there are still some gaps in the literature regarding the role of BDNF in the pathogenesis of OA. Previous research reported sixfold higher plasma BDNF levels compared to the synovial fluid BDNF levels in knee osteoarthritis (KOA) patients in the acute stage of joint inflammatory process and there was a positive correlation between plasma BDNF levels and self-reported pain (87).

Exercise therapy is considered the first-choice non-pharmacologic intervention for OA (5, 21, 94). Regular moderate exercises like strength training or walking are known to ameliorate physical functioning and to reduce pain and disability in people with OA (13, 28). However, the mechanisms of action to explain these beneficial effects on pain and function, are not fully understood (78). One of the hypotheses to explain the beneficial effects of exercise is through an anti-inflammatory effect. Nevertheless, the precise underlying mechanism by which regular exercise therapy exert positive effects on the immune system in OA patients is unknown (9).

The immune system can be influenced via exercise through the release of specific signaling molecules, called exerkines, secreted by several tissues throughout the body. One of the first discovered exerkines are the myokines, which are produced by skeletal muscle cells in response to exercise (17). Myokine IL-6 increases in the bloodstream after muscle contraction and stimulates the release of IL-1 receptor antagonist (IL-1RA) and IL-10 by blood mononuclear cells. As such, muscle-derived IL-6 causes anti-inflammatory effects by promoting immune cells to secrete anti-inflammatory cytokines (17, 52). Remarkably, the acute increase of IL-6 during exercise is not preceded by an increase in pro-inflammatory cytokine TNF-α, which is the case in septic or pathologic conditions (52). Furthermore, the acute and chronic exercise-induced effects differ from each other as resting levels of IL-6 are lower in healthy individuals after chronic exercise as compared to healthy untrained individuals. Accordingly, the exercise-induced effect of IL-6 is divers (i.e. pleiotropic) and context dependent (i.e. acute or chronic exercise) (17). A recent review of the literature concluded that in healthy elderly the levels of pro-inflammatory cytokines (i.e. C-reactive protein (CRP), TNF-α, IL-6) were reduced after an exercise intervention program (6). However, these effects were more heterogeneous in elderly with a specific disorder. Additionally, it was suggested that repetitive exercise (i.e. chronic exercise) can influence the secretory profile of blood mononuclear cells, apparently through the release of myokines. This promotes the secretion of anti-inflammatory cytokines and can consequently counteract inflammaging (6).

On the other hand, there are suggestions that myokines also have a role in the communication between muscles and the brain (72). Centrally, exercise-induced effects are most clearly observed in the hippocampus. It has been demonstrated that the volume of the hippocampus increased in healthy humans after aerobic exercises and after a walking intervention in elderly. This can be explained by the function of BDNF, which levels are increased, on cell proliferation (17, 72). BDNF is expressed after exercises and circulatory BDNF is increased after an acute session of aerobic exercises. Whether BDNF is secreted into the circulation by contracting muscle cells is still unclear (41, 72). It seems that the brain is the main source of circulating BDNF during exercise (71). This raises the question whether other molecules are involved -apparently myokines- which are peripherally secreted and reach the brain/hippocampus to stimulate central BDNF production (17, 72). Nevertheless, BDNF derived by contracting muscles has a metabolic effect on the muscle itself as it will stimulate the oxidation of fat and use of glucose (72). In elderly, a meta-analysis demonstrated that an aerobic program had larger effects on the increase in BDNF levels than resistance training, which also supports the hypothesis that muscle derived BDNF is not released into the bloodstream (24, 71). However, no significant increases in BDNF after exercises were demonstrated as compared to non-exercising elderly (24).

Although exercises have positive effects on OA related symptoms (i.e. pain, disability) (42), the underlying working mechanism of physical exercise is not fully discovered (78). A general overview of acute and chronic exercise-induced effects on inflammation and BDNF in KOA lacks in literature, which is needed to further unravel and understand the underlying exercise effects in the KOA population. Therefore, we aim to summarize all relevant and available literature regarding the effects of physical exercise therapy on inflammatory biomarkers and/or BDNF in patients with KOA in this systematic review. As such, important insights can be discovered, stressed and shared with researchers and clinicians to improve therapy effects in the future.

METHODS

The systematic review protocol was registered at PROSPERO (CRD42020162746) and conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (57).

Information sources
A systematic search was conducted in PubMed, Web of Science and PEDro (last search was performed on May 5th 2021 for all search databases). Additional studies were identified by scanning the reference lists of included articles.

Two reviewers (D.B., E.M.) identified search terms using MeSH vocabulary and text word searching, one reviewer (E.M.) pilot tested the search strategy and discussed afterwards with 2 reviewers (D.B., I.B.) until a final search strategy was developed. Search terms were determined based on PICO dimensions: osteoarthritis (Population), physical exercise (Intervention), inflammatory markers, BDNF (Outcome). The final search strategies can be found in Table 1.

Study selection
Studies had to meet following inclusion criteria:

(1) Studies had to describe the effect of physical exercise on inflammatory markers and/or BDNF.
(2) Randomized controlled trials (RCT), non-randomized controlled trials (NRCT), trials with a pre-experimental design and pilot studies of which a full text was available were included.
(3) Participants of both sexes and any age with OA were considered.
(4) There was no restriction in grade of OA.
(5) At least one exercise intervention had to be investigated in the study. All types of exercise therapy (e.g. aerobic, strength, flexibility,…) were allowed. No restriction was made on duration of intervention and follow-up.

Studies that were written in another language than English, Dutch, German or French were excluded.
Eligibility assessment was independently performed by two reviewers (S.P. and K.L.). Articles were preliminary screened on title and abstract. Thereafter, full texts of the remaining articles were screened, if no sufficient information was provided in the abstract. If needed, authors of the articles were contacted to obtain full texts. Both researchers (S.P and K.L) were not blinded for author details (e.g. names, institution). Disagreements between reviewers were resolved by consensus or by a third independent reviewer (L.L.).

Data collection process
Based on 'The Cochrane Collaboration Data Collection Form' a data extraction sheet was developed by two review authors (D.B. and E.M.) (Supplementary Table 1: Data extraction form). One review author (S.P) extracted the data from the included studies. If necessary, authors of the included articles were contacted by e-mail to obtain specific information (e.g. concerning mean concentrations of inflammatory cytokines, exercise modalities, p-values, 95% confidence intervals (CI)). If authors did not responded, graphs were measured using GetData Graph Digitizer 2.26 (30), were possible, to obtain an estimation of the raw values if they were not published in the article (1, 29, 32, 39, 85, 86).

Risk of bias assessment
The risk of bias was determined by two independent review authors (S.P. and K.L). Conflicts were resolved by a third independent reviewer (L.L.). The risk of bias in RCTs was assessed with 'A revised Cochrane risk of bias tool for randomized trials' (ROB 2.0) (91). The overall risk of bias judgment is classified into three categories, going from low ROB to some concerns, and ending with high ROB. For other study designs than RCTs, the 'Risk of Bias In Non-randomized Studies- of Interventions' (ROBINS-I) (90) was used. Drugs with anti-inflammatory effects, physical activities, health status, nutritional intake, body composition, age and gender were pre-defined as possible confounding factors. Diet, medication and physical activity were pre-specified as potential co-interventions to be taken into account when assessing the risk of bias using ROBINS-I. According to the ROBINS-I tool, the overall ROB judgment is classified into four categories (i.e. low ROB (lowest class), moderate ROB, serious ROB, and critical ROB (highest class)).

Summary measures
The study outcomes were: circulating or intra-articular inflammatory markers and BDNF. To understand the exercise-induced changes in biomarkers, a focus was placed on both acute effects and effects on basal levels. The acute exercise effect was defined as changes in concentrations of biomarkers during and immediately following a bout of exercise (25); i.e. < 24h after an acute bout of exercise (63). The exercise effect on basal levels was defined as changes in concentrations of biomarkers when the acute exercise-induced changes were washed out, e.g. after an overnight resting period (25); i.e. ≥ 24h post exercise (63).

Planned methods of analysis
A meta-analysis was performed when at least two studies investigated the same outcome and inflammatory biomarker. I^2 was calculated to assess heterogeneity, with an I^2 value ≥ 50% being indicative of high heterogeneity, i.e. there is inconsistency among the results of the included studies (40). Subgroup analyses were performed to discover possible reasons of high heterogeneity, if possible (40). Type of exercise therapy, duration of intervention and sampling method were considered for subgroup analyses to reduce heterogeneity. For results that were not incorporated into a meta-analysis, effect-sizes were estimated, where possible. Based on the type of analysis (within or between group), Hedges's gav or Cohen's d effect sizes (ES) were approximated with the assumption of a 0.5 correlation coefficient (55).

Additional analyses
For the meta-analysis of basal effects, mean change from baseline values and standard deviations were used, or calculated when not available (40), for both study groups (i.e. exercise and control group). Mean values were estimated from medians (99), and standard deviations were derived from the standard error and sample size or from the 95% confidence interval (40).

RESULTS

Study Selection
As shown in Figure 1a, the search procedure resulted in 678 unique papers, of which 624 were excluded based on title and abstract. The remaining 54 articles were evaluated full textual, of which 21 articles (1, 3, 4, 7, 29, 32, 33, 38, 39, 47, 51, 58, 59, 65-68, 80, 85, 86, 102) met the inclusion criteria and were finally included.

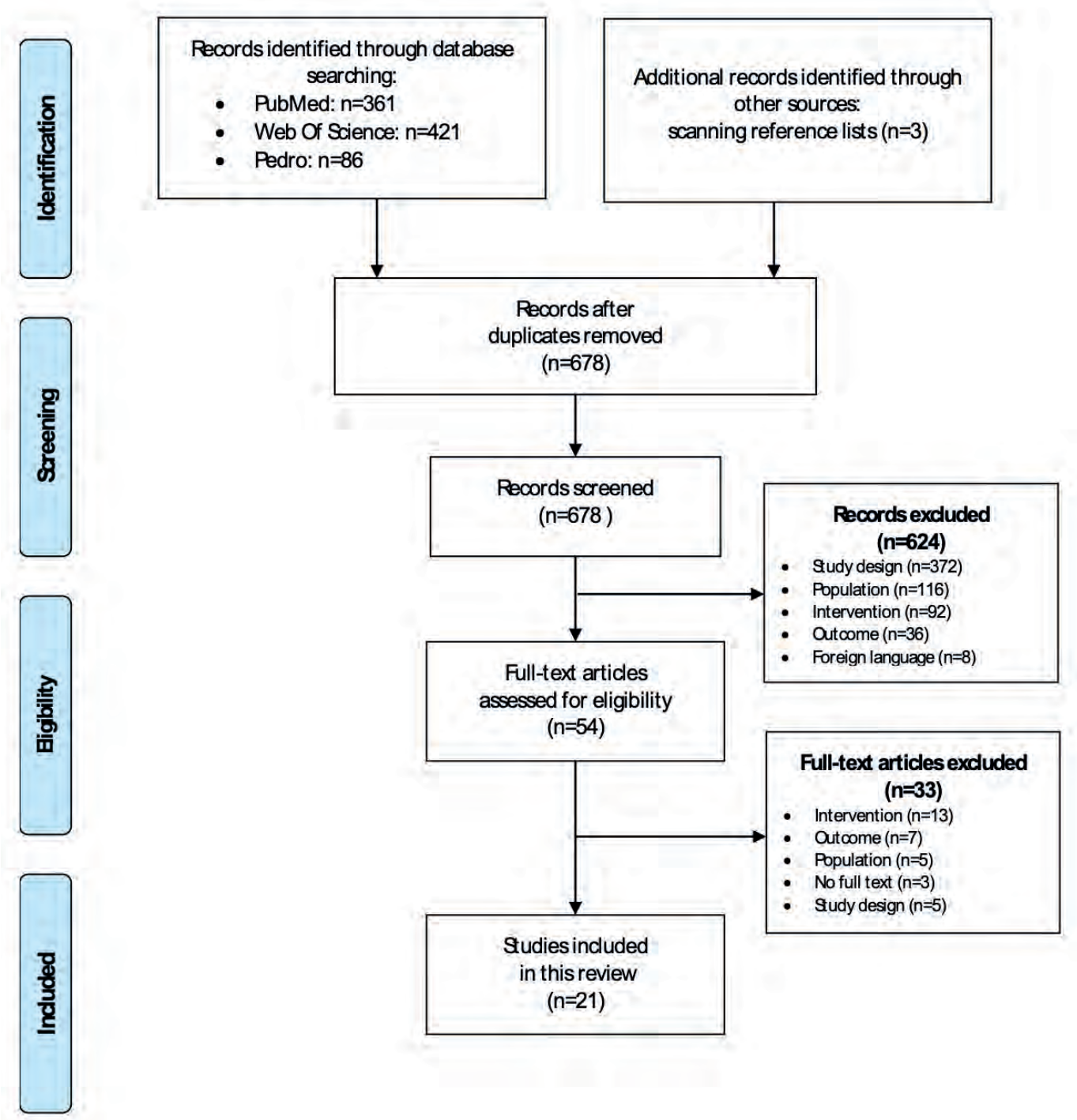

Figure 1a. Flowchart of the study selection

Study characteristics

All included articles were interventional studies (Figure 1b). Fifteen studies were RCTs (3, 4, 7, 38, 51, 58, 59, 65-68, 80, 85, 86, 102) of which two were pilot studies (51, 65). One study was a NRCT (29). Five studies were non-randomized uncontrolled trials (NRUCT) with a pre-experimental design (one group; pre-posttest) (1, 32, 33, 39, 47). Two of them were pilot studies (32, 33). One used a two-phase sequential design (47). Three of the included articles (7, 59, 66) were part of the same trial (64). Two reported on IL-6 (7, 66) while only one reported on CRP (7), and CRP metabolite (CRPM) (59). Therefore, with regard to IL-6, only the results of Messier et al. (66) will be further analyzed in this review as they were only available in detail in this paper.

The included studies involved a total of 1 374 participants, with mean age ranging from 57.57 ± 5.79 years (80) to 75± 7.4 years (85). All studies except two (39, 102) reported on the sex of the participants, as such at least 969 females and 294 males were included in the studies (Table 3) . All 21 articles reported on KOA patients. Only one study also included a healthy control group (i.e. participants without KOA) (29). During the intervention period, at least 287 participants dropped out (in fact several studies did not report the number of blood and/or synovial fluid samples that were acquired during each follow-up and incorporated into the analysis). Supplementary Table 2 provides an overview of all included studies regarding the sample size, number of drop-outs, available samples at each follow-up and how drop-out/data loss was handled in the data analysis.

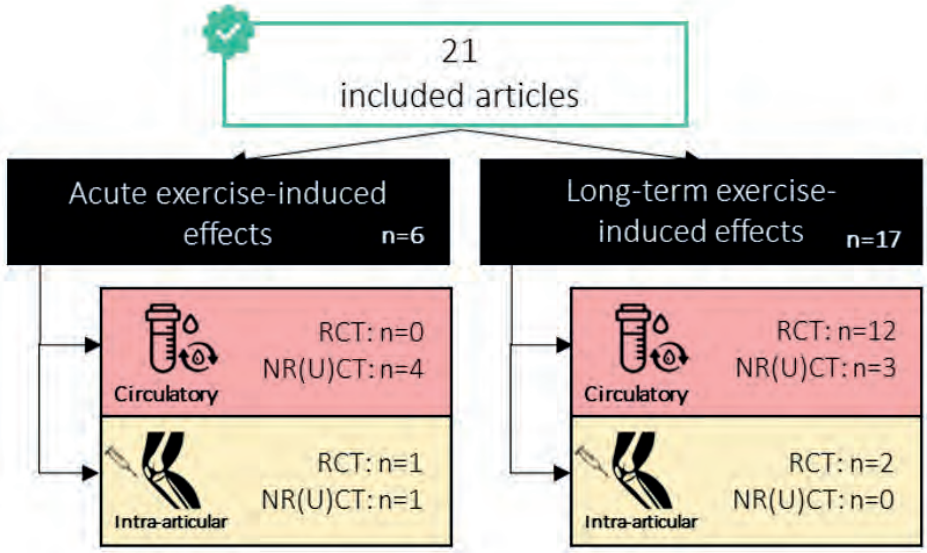

Figure 1b. Overview of the study characteristics. 21 articles were included in this systematic review; 4 focused on acute exercise-induced effects, 15 on long-term exercise-induced effects and 2 on both. A further subdivision of the studies was made based on systemic (i.e. circulatory) or local (i.e. intra-articular) effects, and based on the type of study design.
RCT: randomized controlled trial; NR(U)CT: non-randomized (un)controlled

Eight studies specified overweight and/or obesity as an inclusion criterion (7, 29, 58, 59, 65-68): three articles included patients with body mass index (BMI) between 27 and 40.5 kg/m² (7, 59, 66), three others included patients with BMI ≥ 28.0 kg/m² (65, 67, 68), another article included patients with a BMI ≤ 30.0 kg/m² (58), and one study reported outcomes on 'obese' participants with BMI ranging from 35.9 ± 4.5 kg/m² to 36.2 ± 5.6 kg/m² (29).

Eight articles included KOA patients with a sedentary lifestyle (3, 7, 29, 59, 66-68, 80), three studies included physically active participants (1, 38, 39), and in eight studies physical lifestyle was not defined as an inclusion or exclusion criterion (4, 32, 33, 47, 58, 65, 85, 102). In one study, participants were excluded if they needed a walking aid (86) and in another if they exercised more than 3x/week (51).

The use of anti-inflammatory drugs was handled differently across the studies. In nine studies, medication policy was unclear (1, 4, 32, 33, 47, 51, 67, 80, 86). In four studies, it was allowed to maintain regular medication usage (7, 65, 66, 68). In two of them it was also possible to adjust medication usage during the intervention period (7, 66). In two studies the usage of all pain and anti-inflammatory medication usage was prohibited (38, 39). Five articles reported criteria about gluco-, cortico- or steroid usage (3, 29, 59, 85, 102): in one study, the usage of corticosteroids and/or corticosteroid infiltrations was forbidden (3, 102), in one study glucocorticoids usage was prohibited (85), and in another study steroids were not allowed (29). On the other hand, participants in the study of Loeser et al., 2017, were allowed to use NSAIDs (59). None of the studies adjusted their statistical analysis for concomitant anti-inflammatory drug use.

The included studies were classified into four categories based on the exercise protocols that were investigated: aerobic (n= 5) (3, 32, 33, 47, 80), strength (n=9) (4, 29, 38, 39, 80, 85, 86), aerobic & strength (n=6) (7, 59, 65-68) and other (n=2) (e.g. flexibility training, Tai Chi) (1, 58) (Table 2). Samut et al. compared two types of exercise therapy and was therefore incorporated in both the aerobic and strength category (80).

With regard to the aerobic category, in three studies the exercise intervention consisted of walking (32, 33, 47). In the other study the exercise intervention was cycling and/or swimming (3). In the studies of Gomes et al. 2012 (32) and Gomes et al. 2014 (33), two types of exercise protocols could be distinguished: a training protocol and an acute exercise protocol. The training protocol included a 30 minutes' walk, 3x/week for 12 weeks. The acute exercise protocols of these two studies were identical (walking on a treadmill for 20min with a cool down phase for 30min) (32, 33). The study of Jayabalan et al. (47) consisted of an acute exercise protocol in which participants also had to walk continuously and with intervals of 15 minutes on a treadmill (47). In the exercise phase of the training programs of the included studies in the aerobic group (3, 32, 33, 80) the intensity/load increased progressively during the intervention period. However, small differences are noticeable in training protocols between studies (Table 2).

Exercise protocols of the strength group were more heterogeneous, both in duration of the intervention period and exercise session, intensity/load of the exercises and exercise modality (see Table 2).

In the aerobic & strength category, the exercise protocol of the studies of Beavers et al., 2014 (7), Loeser et al., 2017 (59) and Messier et al., 2013 (66) were the same; as well, the exercise protocol of the studies of Miller et al., 2004 (67) and Nicklas et al., 2004 (68) were identical as they were part of respectively the Intensive Diet and Exercise for Arthritis (IDEA) trial and Arthritis, Diet, and Activity Promotion Trial (ADAPT) study. The exercise protocols of the IDEA and ADAPT studies consisted of an 18 month intervention with a 3 day per week exercise program for 1h, containing an aerobic phase, a strength phase, a second aerobic phase and a cool down phase. The pilot study of Messier et al., 2000 reported a warm-up phase and an intervention period of 6 months (65).

Two studies were categorized as other (1, 58). Aguiar et al., 2015 used an exercise protocol consisting of strength and flexibility training of the muscles (1). The exercises in

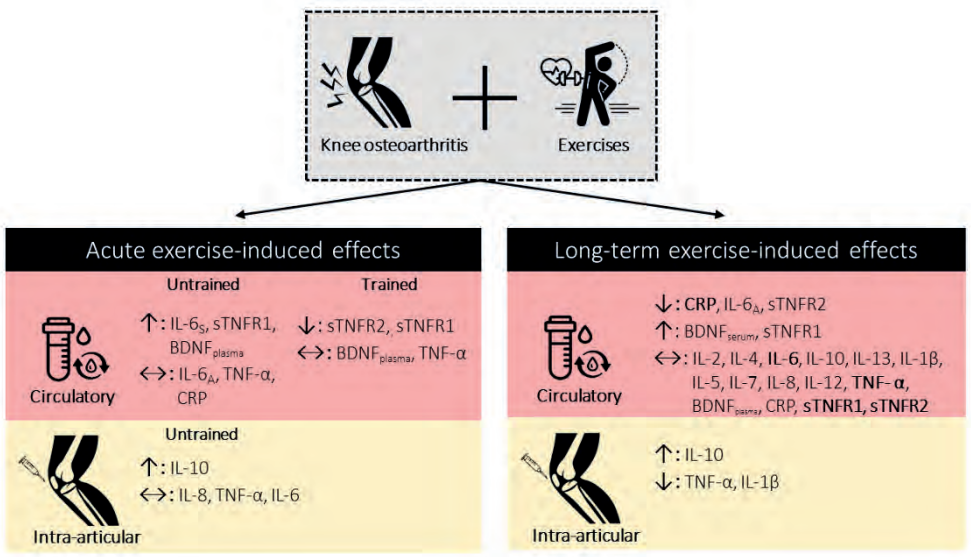

Figure 2. Summary of the main exercise-induced effects in KOA patients.
A division was made between acute and long-term exercise effects, and between circulatory (i.e. blood serum/plasma) and local (i.e. synovial fluid) levels.
'↑' indicates an increase , '↓' indicates a decrease, '↔' indicates no change, 'biomarkers written in **bold***' indicates evidence based on meta-analysis, 'biomarkers normally written' indicates evidence from individual studies.*
A: aerobics; S: strength training; IL: interleukin, sTNFR: soluble tumor necrosis factor receptor, BDNF: brain derived neurotrophic factor, TNF: tumor necrosis factor, CRP: C-reactive protein

the study of Liu et al., 2019, consisted of a warm-up phase, followed by either cycling (aerobics) or Tai Chi or Baduanjin, and ended with breathing techniques and a relaxation phase (58). Detailed information about the exercise protocols of each study is provided in Table 2.

The 18 investigated inflammatory markers were: IL1-β, IL-2, IL-4, IL-5, IL-6, IL-6sR, IL-7, IL-8, IL-10, IL-12, IL-13, TNF-α, soluble tumor necrosis factor receptor (sTNFR)-1, sTNFR-2, BDNF, CRP, CRPM and leptin. Seven studies reported results of biomarker analyses in peripheral blood plasma (3, 7, 32, 33, 66, 85, 86); nine in serum (1, 4, 29, 47, 58, 59, 67, 68, 80); in one study it was unclear whether the biomarkers were analyzed in serum or plasma (51); and four in dialysate of synovial fluid of the knee (38, 39, 65, 102). Four studies investigated the acute effects of exercise on inflammatory markers (29, 38, 39, 47), fifteen studies investigated the effects of exercise on basal levels (1, 3, 4, 7, 51, 58, 59, 65-68, 80, 85, 86, 102), and two studies investigated both the acute effect and the effect on basal levels (32, 33) (Figure 1b). Detailed information is available in Table 3.

Risk of bias within studies

Information regarding the quality assessment of the studies is provided in Table 4.

Randomized controlled trials

The quality of fifteen articles was determined with the ROB 2.0 tool (Table 4a). In eight included RCTs, there were "some concerns" in at least one risk of bias domain (3, 38, 58, 59, 65, 68, 85, 86). Seven RCTs had an overall "high ROB" because there were either "some concerns" for multiple domains or a "high ROB" in at least one domain (4, 7, 51, 66, 67, 80, 102) (Table 4a).

Only two RCTs provided detailed information on the randomization process (domain 1) (59, 68). In two RCTs, a "high ROB" arising from the randomization process (domain 1) was reported, as insufficient information about the allocation sequence concealment was available (7, 80). In two RCTs, the ROB due to deviations from the intended interventions (domain 2) was also "high" (7, 66) while most of the RCTs had a "low ROB" in this domain (3, 38, 58, 65, 68, 85, 86). In all RCTs (n=14), all outcome data were available for all randomized participants (domain 3), except for the study of Miller et al. (67). Apart from one study (51), all RCTs showed a "low ROB" regarding the measurements of the outcomes (domain 4).

Non-randomized or non-controlled trials

The quality of six articles was determined with the ROBINS-I tool (Table 4b). The NRCT of Germanou et al. and the NRUCT of Helmark et al., 2012 had both a "critical overall ROB" score on the ROBINS-I, because both articles had a critical ROB score in domain 5 (bias due to missing data) (29, 39). Four NRUCTs had a "serious overall ROB" score because there was a serious ROB score reported in at least one domain of the ROBINS-I (1, 32, 33, 47).

Exercise effects

Despite the association of inflammation with the pathogenesis of KOA, very few studies have investigated the impact of physical exercise on inflammatory mediators in patients with KOA. This section is further divided into two main parts: acute exercise effects and long-term exercise effects. Studies were allocated to the acute exercise effects section if changes in biomarker concentrations were captured during and immediately following a bout of exercise (i.e. < 24h after an acute bout of exercise). Studies were allocated to the long-term exercise effects section if changes in biomarker concentrations where captured > 24h post exercise (i.e. when the acute exercise effects were washed

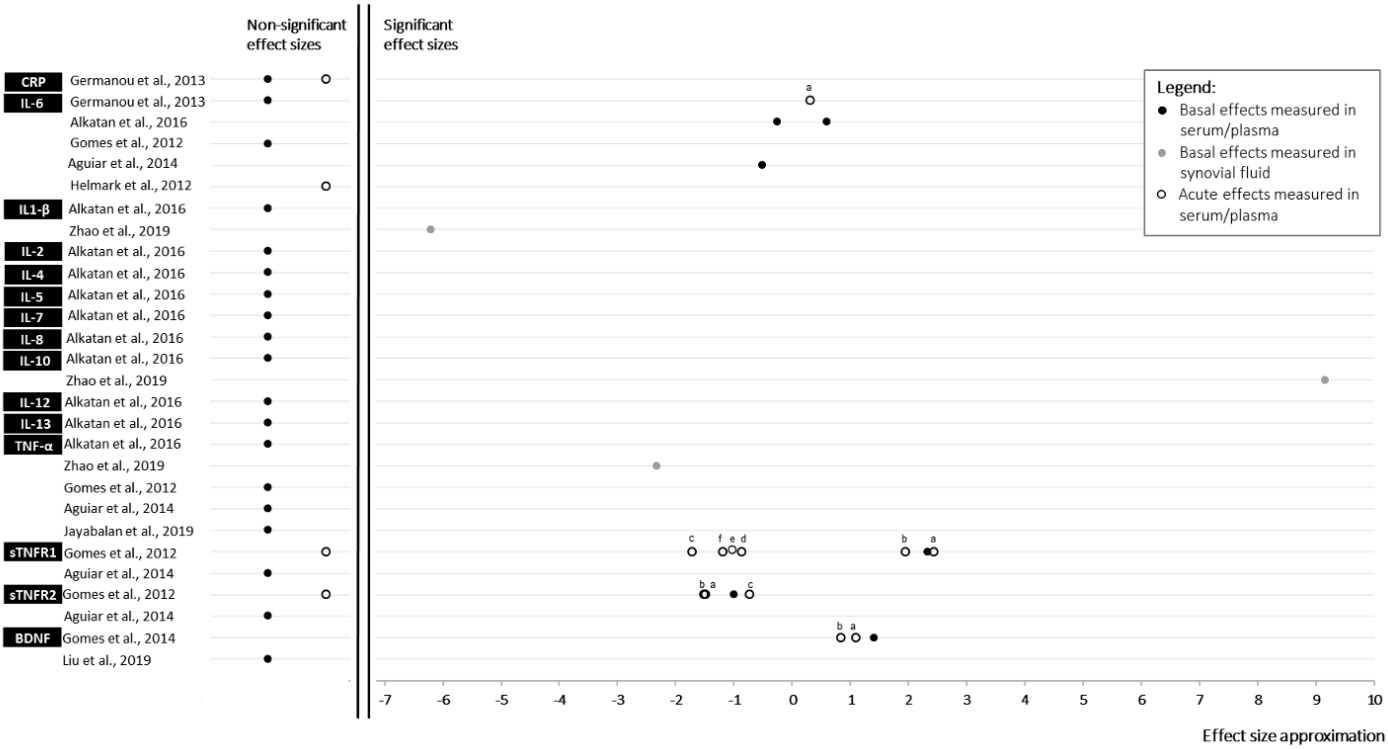

Figure 3. Impact of exercise therapy on different biomarkers in KOA patients (studies not incorporated into meta-analysis).
[a]: in untrained status pre vs. post exercise session. [b]: in untrained status pre vs. 30min post exercise session. [c]: in untrained status pre vs. 30min post exercise session. [d]: in trained status post vs. 30min post exercise session. [e]: post exercise session in untrained status vs. post exercise session in trained status. [f]: 30min post exercise session in untrained status vs. 30min post exercise session in trained status.

out). Detailed information of the individual study results can be found in Table 3 and a schematic overview of the main results is presented in Figure 2 and 3. Table 5 provides a summary of the exercise effects per biomarker. The biomarkers are referred as pro or anti-inflammatory based on their characteristics and context in which they were measured. Based on literature data, TNF-α, CRP, IL-1β (61, 96) can be considered as pro-inflammatory biomarkers, while IL-10, IL-4 and sTNFR1&2 as anti-inflammatory biomarkers (61, 87). IL-13 can also be considered as anti-inflammatory as it inhibits the expression of pro-inflammatory biomarkers (e.g. TNF-α, IL-8) (46). Depending on the context IL-6 can induce pro or anti-inflammatory effects and is therefore considered as a pleiotropic cytokine (17). When measured within 24 hours after an exercise session, circulating IL-6 is mainly secreted by the exercising muscles and has mainly anti-inflammatory properties. When measuring basal levels of IL-6, > 24 hours after an exercise session, circulating levels reflect rather pro-inflammatory status (17). Circulatory IL-5 is mainly released by a Th2 immune response to activate and recruit eosinophiles and basophiles (101). Leptin is also categorized as a biomarker with a pro-inflammatory character as it increases the expression of other pro-inflammatory cytokines in the circulation (e.g. TNF-α, IL-6) (44). Chemokines like circulatory IL-8 are pro-inflammatory because of the attraction of immune cells to the inflammation site and IL-8 induces matrix metalloproteinase (MMP) activity (12). IL-8 is also involved in increased capillarization response following exercise and might therefore also reflect beneficial signaling (26) Intra-articularly, macrophages secrete pro-inflammatory cytokines like IL-12 to initiate a Th1 response (16). Also intra-articular IL-7 is considered as a pro-inflammatory cytokine in OA. It is secreted by chondrocytes and induces cartilage destruction. Furthermore, it may initiate a T-cell driven immune response (95).

Acute exercise effects

Effects measured in serum or plasma

The circulatory blood concentrations of the pro-inflammatory biomarkers CRP (ES: 0.052) (29) and TNF-α (ES: -0.019; ES: 0.081) (32, 47) in patients with KOA did not change after a single bout of exercise. Only after a single bout of strength training, an increase in IL-6 concentration was demonstrated (ES: 0.314) (29), as IL-6 did not change after an acute session of aerobic training (32). Furthermore, the sTNFR1 (ES: 2.444) (32) and BDNF (ES: 1.101) (33) levels were increased immediately after one aerobic session in untrained condition, while a decrease was noticed for sTNFR2 (ES: -1.483) (32). In trained condition (following a 12 week aerobic training program), both sTNFR1 (ES: -1.714) and sTNFR2 (ES: -0.727) levels significantly decreased 30 minutes after an acute exercise session, while an acute training session in trained condition had no significant effect on BDNF (33).

In summary, an acute exercise session elicited an acute myokine response (ES IL-6: 0.314), as well as a decrease in sTNFR2 (ES: -1.483) and increase in BDNF (ES: 1.101) in untrained condition. Myokine response seems more pronounced in strength training compared to aerobic training, as well as in trained condition (ES sTNFR1: -1.714; ES sTNFR2:-0.727). No evidence was found for an acute pro-inflammatory effect (ES CRP: 0.052 and ES TNF-α: -0.019 and 0.081) following an acute bout of training.

Effects measured in synovial fluid

No difference in the levels of intra-articular inflammatory biomarkers (TNF-α, IL-8, IL-6 and IL-10) was found following exercise versus control. Due to the invasive repetitive sampling procedure (6 samples between 1 and 3 hours following exercise or control), the inflammatory biomarkers increased

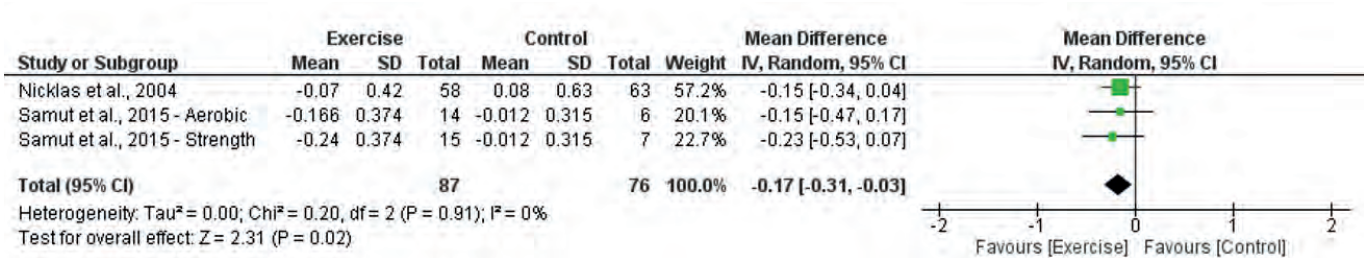

Figure 4. Forestplot of comparison exercise vs. control for basal CRP levels.

similarly in both groups, except for IL-10. This anti-inflammatory biomarker did only increase after the exercise condition. In another study, intra-articular IL-6 levels, measured 10-15 minutes after a strength exercise showed a tendency to increase (ES=0.900), but this effect was not statistically significant.

In summary, a single bout of exercise elicited an intra-articular anti-inflammatory response as IL-10 increased. No evidence for an acute exercise induced intra-articular inflammatory response was demonstrated.

Long-term exercise effects

Effects on pro-inflammatory markers measured in serum or plasma

For three pro-inflammatory markers in the blood (i.e. CRP, IL-6 and TNF-α), a meta-analysis was performed (Figure 4 -6) by combining results of two studies (68, 80). Basal CRP levels decreased (mean difference: -0.17, 95% CI -0.31 to -0.03; p=0.02; I^2=0%; p= 0.91) while IL-6 (mean difference: 0.21, 95% CI -0.44 to 0.85, p= 0.52; I^2=61%, p=0.08) and TNF-α concentrations (mean difference: -0.57, 95% CI -1.47 to 0.32, p=0.21; I^2=71%, p=0.03) did not change in KOA patients after an intervention period ranging from 6 to 18 weeks in comparison to the control group. Those studies that could not be included in the meta-analysis showed that, an exercise program of either cycling (ES: -0.259), swimming (ES: -0.596), or flexibility training in combination with strength exercises (ES: -0.513) induced a significant decrease in circulatory levels of IL-6 (1, 3). Additionally, swimming or cycling for 6 weeks did not impact other pro-inflammatory cytokines in the blood circulation of KOA patients (i.e. IL-1β, IL-5, IL-7, IL-8, IL-12, TNF-α) (3). On the other hand, no changes in IL-6 (SMD: log (IL-6): -0.02 [95%CI: -0.32 to 0.28]), TNF-α (SMD: log(TNF-α): 0.10 [95%CI: -0.07 to 0.26]) and CRP (SMD: log(CRP): -0.25 [95% CI: -0.69 to 0.20]) were demonstrated when KOA patients performing strength exercises for 4-6 weeks were compared with a true KOA control group (51). As well, a single bout of strength training had no influence on the basal IL-6 (ES: 0.035) and CRP (ES: 0.039) levels when measured 24h after the exercise (29). Furthermore, no significant changes in IL-6 (ES:-0.278) and TNF-α (ES: 0.546) were observed when KOA patients performed aerobic training for 12 weeks (32), neither TNF-α (ES: 0.080) was significantly influenced after flexibility training in combination with strength exercises (1), and IL-1β was also not significantly changed in patients with KOA who underwent a strength intervention (ES: 0.146) (4).

Effects on anti-inflammatory markers measured in serum or plasma

Besides pro-inflammatory markers, anti-inflammatory cytokines (IL-2, IL-4, IL-10, IL-13, sTNFR1, sTNFR2) were also investigated in blood serum or plasma of patients with KOA (3, 68, 85). The results of two studies (68, 85) were combined into a meta-analysis for sTNFR1 and sTNFR2 (Figure 7-8). No significant changes in basal sTNFR1 (mean difference: -34.94, 95% CI -125.60 to 55.72, p=0.45, Figure 7) and sTNFR2 (mean difference: -214.27, 95% CI -769.99 to 341.15, p= 0.46, Figure 8) levels were found in KOA patients after an intervention period ranging from 12 weeks to 18 months in comparison to the control group. However, after a 12 week aerobic intervention in woman with KOA, sTNFR1 (ES: 2.325) was increased while sTNFR2 (ES: -0.997) was significantly decreased (32). Other anti-inflammatory markers (i.e. IL-2, IL-4, IL-10, IL-13) did not significantly change in patients with KOA after an intervention period of either swimming or cycling (3).

Effects on BDNF measured in serum or plasma

Results of basal BDNF were available in two studies (33, 58). No significant changes in serum BDNF levels were noticed after 12 weeks when comparing the three intervention groups, namely Tai Chi (ES: -0.370), Baduanjin (ES: -0.512), aerobics (ES: -0.185), with the control group (58). On the other hand, an increase in plasma BDNF (ES: 1.412) was demonstrated in female participants with KOA who performed a walk training of 12 weeks (33).

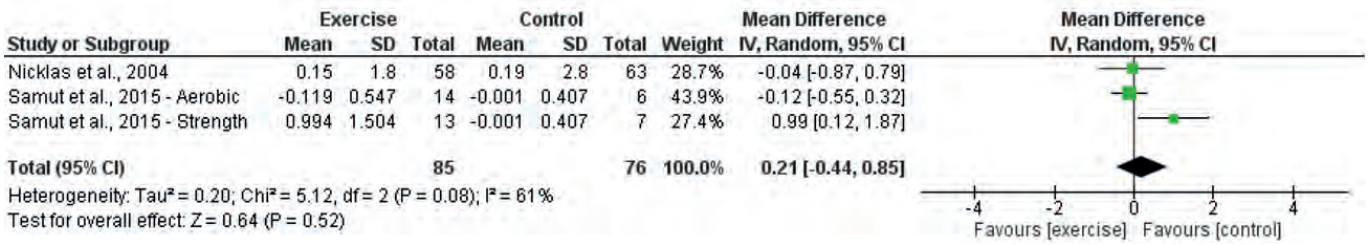

Figure 5. Forestplot of comparison exercise vs. control for basal IL-6 levels.

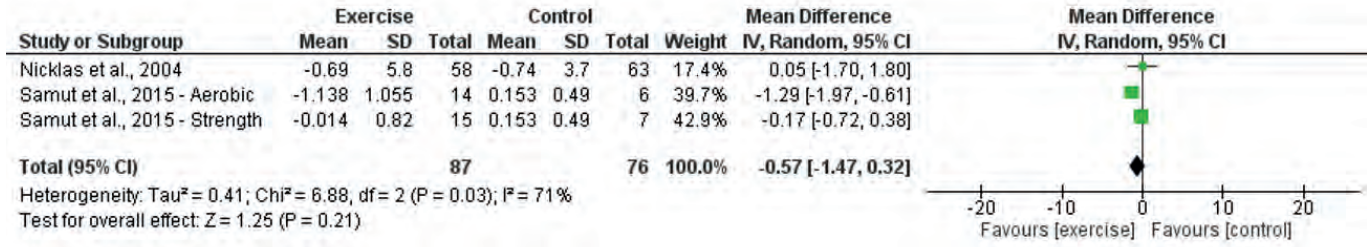

Figure 6. Forestplot of comparison exercise vs. control for basal TNF-α levels.

In summary, our meta-analysis showed that basal CRP can be reduced in patients with KOA through an exercise intervention of 6-18 weeks, while IL-6 and TNF-α levels did not significantly change. Also, sTNFR1/2 did not significantly change after at least 12 weeks exercising. The exercise-effect on different other biomarkers was less clear and, unfortunately, there were insufficient data available to perform a meta-analysis. Nevertheless, a low degree of evidence was present for a decrease in IL-6 after swimming (ES: -0.596), cycling (ES: -0.259) or flexibility training in combination with strength exercises (ES: -0.513) for 12 weeks, increase in sTNFR1 (ES: 2.325), decrease in sTNFR2 (ES: -0.997) and increase in plasma BDNF (ES: 1.412) after 12 weeks of walking.

Effects measured in synovial

Intra-articular, strength training of 12 months decreased basal levels of TNF-α (ES: -2.322) and IL-1β (ES: -6.199), and increased the levels of anti-inflammatory cytokine IL-10 (ES: 9.163) (102). Additionally, a combined exercise and diet program of 6 months decreased the IL-1β synovial fluid levels significantly (65).

In summary, we found evidence that a strength intervention with static low angle squats can significantly counteract local inflammation in the knee reflected by increased intra-articular levels of IL-10 (ES: 9.163), and reduced IL-1β (ES: -6.199) and TNF-α (ES: -2.322).

DISCUSSION

This systematic review provides an overview of studies investigating exercise-induced effects on inflammatory markers and BDNF in patients with KOA, with a focus on both acute and basal circulatory and/or intra-articular effects. Overall, our literature study provides some evidence that exercise therapy in patients with KOA can elicit circulatory and intra-articular anti-inflammatory effects, on the long-term as well as after a single exercise session. Twenty-one articles were included (acute: n=4; basal: n=15; both: n=2) that reported on eighteen different inflammatory markers and BDNF. For five biomarkers (CRP, TNF-α, IL-6, sTNFR1 and sTNFR2) a meta-analysis was performed, to investigate the long-term exercise induced effects. Due to limited available data of the other biomarkers, no meta-analysis could be performed but effect sizes were estimated (where possible).

CRP is an important biomarker that reflects circulatory inflammation as it is produced, after induction (primarily) by IL-6, in the acute phase of the inflammatory process in the liver (96). CRP concentrations did decrease after a chronic exercise intervention program in KOA patients compared to control patients with KOA which did not perform exercises (MD: -0.17; 95% CI: -0.31 to -0.03) (Figure 4), while one single bout of exercise did not change basal and acute CRP levels (29). These findings suggest that one single bout of exercise is not effective to decrease basal CRP concentrations in patients with KOA, but it seems effective to reduce resting blood CRP concentrations after maintaining exercise therapy, leading to an anti-inflammatory effect on the longer term. These results are in line with two recent meta-analyses which reported a significant reduction in basal CRP levels after an aerobic or strength training exercise intervention in older adults (81, 103).

Because basal levels of CRP decreased, we expected that IL-6 levels would also decrease in patients with KOA in response to exercise therapy, though this was not confirmed by our meta-analysis (Figure 5). One of the studies incorporated in the meta-analysis (80) reported that the sample size of their study was too small (n=42) and the intervention period of 6 weeks was too short to demonstrate significant changes in the investigated inflammatory markers (80). Two other included articles in this review, with a pre-experimental study design (without control group), reported a decrease in circulatory IL-6 levels (1, 3). Noticeably, the intervention period of both studies was twice as long as the study of Samut et al. (80). Accordingly, it seems that an exercise therapy program of at least 12 weeks is necessary to achieve such an anti-inflammatory response on the long-term; however, this needs confirmation by high-quality RCT's.

Effects of an exercise intervention can interact with

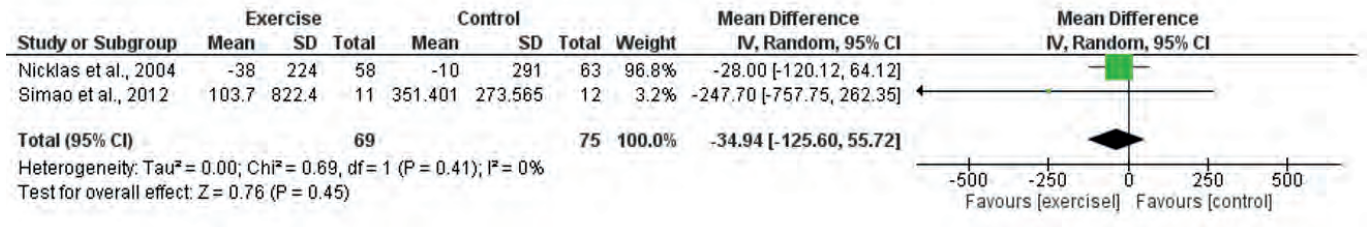

Figure 7. Forestplot of comparison exercise vs. control for basal sTNFR1 levels.

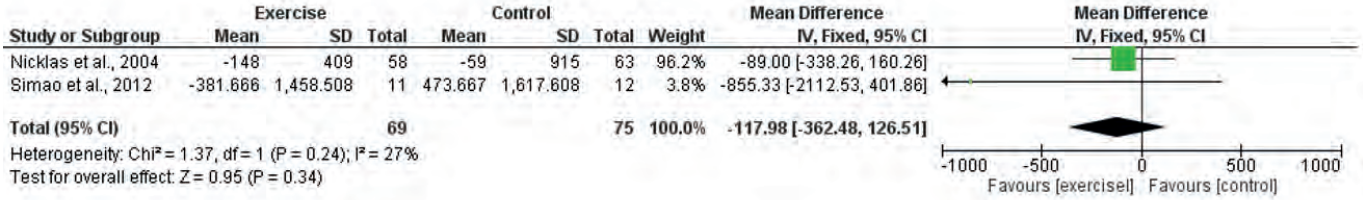

Figure 8. Forestplot of comparison exercise vs. control for basal sTNFR2 levels.

changes in fat and body composition. Germanou et al. (29) observed significantly higher resting IL-6 levels in participants with KOA at baseline in contrast to control participants with healthy knees. This study included only obese female participants with KOA (29). To explain the higher resting levels in OA patients, the link between obesity and inflammation should be considered. White adipose tissue can function as a key endocrine organ by releasing multiple pro-inflammatory adipokines (leptin, chemerin, visfatin etc.) and cytokines (IL-6, TNF-α, etc.) and may contribute, mediate or interact with the inflammatory process (27, 50, 83). At the level of the knee joint, the infrapatellar fat pad also produces locally cytokines (e.g. IL-6, IL-8, TNF-α) and adipokines (e.g. visfatin, adiponectin, adipsin), and stimulates the progression and development of synovitis that contributes to the pathophysiological changes in the KOA process (48). Certainly, obesity is one of the risk factors for OA because of the associated local effects (i.e. higher joint loading) and systemic effects (i.e. inflammation) (76). Almost half of the included studies in this review were patients with obesity. We also included studies that investigated the additional effect of a diet intervention besides an exercise intervention in patients with KOA. However, we focused only on the results of the exercise intervention in this systematic review. Those studies showed that a diet program was more effective to reduce resting leptin (67), IL-6 (66, 68), sTNFR1 (68), CRP (7, 68) levels than exercise therapy. In this respect, it should be noted that interventions focusing solely on weight loss as a treatment for OA may negatively impact muscle mass and strength, and consequently, mobility (11). Accordingly, it is important to propose a weight loss program in combination with exercise therapy for overweight or obese patients with OA; which is also stated in the American College of Rheumatology guidelines (11).

Sex is also an important risk factor for KOA, besides obesity. In general, the prevalence of KOA is 21.7% in women and only 11.9% in men (19). These percentages can explain why the studies in our systematic review mainly included female participants (i.e. about 80%). In general, male KOA study participants were strongly underrepresented (only 20%) in the included articles. In six articles (4, 29, 32, 33, 38, 86), the study participants even consisted only of females. It is known that females experience OA in a different way than males. For example, females suffer from a higher degree of KOA symptomatology, which is reflected in higher levels of disability and pain (35). KOA can have a multifactorial origin, but hormonal changes, previous knee injury and anatomy of the knee are the most important ones (35). Especially during the menopause, females experience a decrease in oestrogen. In fact, oestrogen has protective properties on the cartilage, and these protective properties seem to decline in the post-menopausal period (35). Strikingly, none of the included studies compared neither baseline nor exercise-induced changes in inflammatory biomarkers between male and female participants. Therefore, it remains unclear what the influence of sex can be on the expression of certain inflammation related biomarkers after exercise therapy. This shortcoming needs to be addressed in future trials to further clarify possible differences between males and females, as suggested by Hame and colleagues (35).

Another pro-inflammatory cytokine of interest was TNF-α and its soluble receptors; sTNFR1 and sTNFR2. TNF-α is one of the key cytokines involved in the initiation of the immune response. The soluble TNFRs function as inhibitors of TNF-α as they can compete with TNF-α for binding to the cell surface TNFRs (85, 98). According to Simao et al., a reduction in sTNFR1 and sTNFR2 levels may reflect anti-inflammation and reduces the inflammatory process (85). In this systematic review, our meta-analysis (Figure 7-8) demonstrated that there were no significant reductions in basal levels of sTNFR1 and sTNFR2 after exercise therapy in patients with KOA. However, for both receptors, a trend to decrease in mean difference was observed in favor of the exercise therapy groups but this decrease was not statistically significant. Furthermore, the studies that investigated the exercise-induced effects on basal TNF-α levels did not observe a significant change after the intervention (1, 3, 32, 51, 68, 80). This finding is in line with results of studies that investigated the effect of exercise in populations without OA. For example, evidence of a large-scale study in a non-disabled elderly population at risk for physical disability showed that a 12-month exercise intervention did not result in significant changes of circulatory TNF-α; however, they did observe a significant decrease in sTNFR1 and IL-6, but not in sTNFR2 (8). It is important to highlight that capturing blood concentrations of TNF-α using immunoassays is not evident since this cytokine has a short half-life of 4.6 min. Circulatory sTNFR1 and sTNFR2 are more stable and can therefore provide an indication of the active TNF-α signaling during inflammatory conditions (85, 88).

The neurotrophin BDNF was investigated in two studies with contradictory results (33, 58). Liu et al. (58) measured serum BDNF while Gomes et al. (33) measured plasma BDNF. Plasma levels of BDNF reflect the free active circulatory BDNF while serum BDNF indicates the total BDNF concentration (i.e. it is the sum of stored BDNF within the platelets + plasma BDNF) (97). As such, serum BDNF can show variations in BDNF levels over a longer time whereas plasma BDNF gives temporary concentrations. Moreover, it is more appropriate to measure BDNF in blood serum instead of plasma because plasma BDNF levels are unstable (≤ 1 hour present) (75, 84). Measurement of plasma BDNF can also be affected by sample handling as BDNF can be released from platelets, that are present in the blood tube, when activated (75). Based on this knowledge, we agree that the determination of serum BDNF is more correct to show long-term exercise induced effects in patients with KOA. Accordingly, no

significant changes in serum BDNF were observed in patients with KOA after 12 weeks of Tai Chi, Baduanjin or cycling (58). This result is in contrast with others as they found an increase in BDNF in healthy elderly after exercise therapy (15, 18). However, circulatory BDNF in OA is increased as compared to healthy controls (70). These elevated levels of BDNF can play a role in central sensitization, (70) which is present in some patients with KOA and can be an explanation for chronic pain complaints (69, 70).

Besides long-term circulatory effects of exercise therapy, local effects in the knee were investigated by Zhao et al. which determined synovial fluid levels (102). Their results were promising as static low angle squats were effective to reduce local inflammation (i.e. synovitis) in the knee joint space of people with KOA. Messier et al. did not report on the IL1-β levels of the exercise group alone, but they demonstrated a decrease in synovial fluid levels of IL-1β in eight patients with KOA that followed a weight loss program in combination with exercise therapy (65). To our knowledge, no similar clinical research on long- term effects in humans is present in the literature, but the results are also comparable with pre-clinical research. During exercise, repeated mechanical loading is provoked within the knee. In-vitro studies demonstrated that IL-1β induced biomarkers are downregulated when chondrocytes are mechanically stimulated. Furthermore, inflammatory responses related to IL-1β and TNF-α are counteracted upon chondrocyte stimulation (56). In-vivo, it was shown that IL-10 was synthesized in menisci of rabbit knees when passive motion therapy was applied (56). Further research on the long-term effects of exercise therapy in patients with KOA and healthy individuals is necessary to further elucidate the immune response at the local level of the knee.

Little is known about how exercise-induced effects on inflammation related biomarkers in the synovial fluid are related to circulatory biomarkers in patients with KOA, and vice-versa. On the one hand, it is unclear to which extent exercise-induced changes in systemic inflammation contributes to or reflects changes in intra-articular inflammatory processes in patients with KOA. On the other hand, in previous research (9), different hypotheses on the working mechanism of exercise therapy in KOA patients are suggested. One of the hypotheses proposed that exercise therapy improves the blood circulation and allows the synovial fluid to move, causing an outflow of inflammation related markers from the joint cavity (9). Unfortunately, there is a lack of data available and future research is needed to confirm these hypotheses. In this systematic review, we did not identify articles investigating both systemic and intra-articular effects on inflammation related biomarkers. The study of Helmark et al. (39) already initiated this investigation as cartilage oligomeric protein (COMP) was determined in both serum and synovial fluid. However, for inflammatory related biomarkers like IL-6, the determinations were only performed in synovial fluid (39). In general, studies investigating biomarker levels in synovial fluid are very challenging as intra-articular fluid is very limited (49). On the other hand, our systematic review was able to provide an overview of inflammation related biomarkers in the synovial fluid and provides important new insights to the research community.

A distinction between acute exercise-induced effects and long-term effects was made because certain cytokines, especially pleiotropic cytokine IL-6, have properties that differ from an acute or basal setting. Acute circulatory levels of IL-6 can have anti-inflammatory effects, while elevated resting levels rather reflect a pro-inflammatory status. Elevated blood levels of IL-6 immediately after exercising are mostly derived from contracting skeletal muscles. This myokine release is dose-dependent with a higher release seen after more intensive and/or longer muscle activity (22). This relationship can also be substantiated to the KOA population as only after an acute bout of strength training, a significant increase in IL-6 was observed in patients with KOA, but this effect washed-out after 24 hours (29). Furthermore, a decrease in acute levels of sTNFR1 and sTNFR2 was observed in trained condition, while this was only the case for sTNFR2 in untrained condition (32). Indeed, IL-6 induces sTNFRs release and regulates the TNF-α levels (74). However, for BDNF an increase was only observed in untrained status (33). Nevertheless, this finding is based on one study, therefore future investigations are needed. No indications for exacerbation of inflammation were found after an acute bout of exercise, as circulatory CRP and TNF-α levels did not change. Only six articles investigated the acute exercise-induced effects. Literature regarding acute effects on circulating anti-inflammatory cytokines such as IL-10 and IL-1RA is lacking. However, intra-articularly, IL-10 did significantly increase after a single bout of exercise (38). A small pilot-study in which five healthy young men performed a single running session of 30 minutes also suggested that an acute bout of running can decrease intra-articular inflammation (43). However, to our knowledge, this is the only clinical study available in literature that investigated a wide set of biomarkers in synovial fluid in healthy individuals who performed an acute exercise session. Studies with a larger sample size are recommended to investigate the acute intra-articular response on exercise.

The findings of this systematic review imply that exercise therapy for people with KOA can trigger anti-inflammatory reactions, making this intervention effective and accessible to control the inflammation (i.e. synovitis and CLIP) associated with KOA. However, a proportion of the patients with KOA still believe that exercise therapy or physical activity is dangerous and will cause harm (89). To overcome such incorrect beliefs, pain science education can be an added value (89), and the findings of the present review and meta-analysis suggests that such pain science education for patients with KOA should be updated with information about the anti-inflammatory properties of exercise therapy. A large RCT in which people with OA of the lower extremities (i.e. knee, hip, lower back, ankle, feet OA) performed strength training for 8 weeks in combination with 30 minutes education sessions (in which the management of barriers to exercise and strategies to exercise safely within OA are covered) showed a significant decrease in serum TNF-α post intervention (62). Furthermore, a recent systematic review of RCTs concluded that exercise is safe for patients with KOA (14).

Here, we summarize the strengths of this systematic review. First, we reported the findings according to the PRISMA guidelines. Second, multiple independent blinded researchers performed the study selection and quality assessments. For the study selection, a predefined set of in and exclusion criteria were determined and this protocol was published on PROSPERO (CRD42020162746) prior to final database search, study inclusion and data extraction. Regardless of the author details

of the papers (e.g. name and/or institution), the quality of the studies was rated. As such, author details had no influence on the results of the systematic review. Furthermore, the quality assessments for the RCTs were performed using the most recently developed ROB 2.0 tool (91). Third, a meta-analysis of five different biomarkers of interest was performed. Effect sizes of results that could not be incorporated into a meta-analysis were estimated where possible, allowing easier comparison of the results of those studies. Finally, this is the first systematic review that provides a comprehensive overview of acute and basal exercise-induced effects on inflammatory biomarkers in KOA patients (measured in serum, plasma or synovial fluid). The systematic review of Bricca et al., does not include acute exercise- induced effects and it seems that they made no distinction between systemic and intra-articular effects (14). Furthermore, these authors only focused on one type of study design, RCTs (14). To distinguish systemic from local effects, it is essential to measure biomarkers respectively serologically or intra-articularly. Levels of systemic markers result from multiple tissues or joints throughout the body, whereas synovial fluid focusses only on the state of one joint (20). In fact, the (anti)inflammatory response to exercise may differ in the peripheral circulation compared to the synovial fluid.

This systematic review has also some limitations. First, the included studies reported on different cytokines. Therefore, it was not possible to perform a meta-analysis for every biomarker studied. Furthermore, a low number of studies reported on acute exercise effects. Additionally, included studies reported their results in various ways (e.g. only graphs, narrative descriptions, or quantitative descriptions). When quantitative data were not reported and authors did not respond to our request for these data, we performed additional analyses in order to obtain the best possible estimates. Graphs were analyzed with GetDataDigitizer as accurate as possible to obtain an estimate of the effects. Furthermore, to perform a meta-analysis, mean values were required. With the use of mathematical formulas (40, 99), mean values were approximated if they were not available. However, these method comprises risks for errors. Third, the study designs of the included studies were heterogeneous. More high qualitative studies, preferably RCTs that investigate both acute and basal exercise-induced effects on a wide set of inflammatory markers and BDNF, are needed to overcome this limitation and further extrapolate potential underlying relationships between acute and basal exercise-induced effects. To investigate the differences between different types of exercise therapy (i.e. strength, aerobics, flexibility training etc.), interventional studies that determine the same set of biomarkers and BDNF serologically and intra-articular are needed in people with OA. As such, it can be demonstrated whether there is a difference in inflammatory response between those types of exercise therapy because there was lack of data on this topic. Finally, per definition, reviews are limited by the quality of the available research reports. Unfortunately, sleep is not considered as a potential confounder of studies examining the exercise effects on inflammation in patients with KOA. This is a shortcoming that needs to be addressed in future work. Indeed, insomnia is a severe and very common comorbidity in patients with KOA (2, 92, 100), and it has a dysregulating role on inflammatory pathways in the general population as well as in KOA patients, with increases in markers of systemic inflammation such as IL-6 and CRP (23, 37, 45).

CONCLUSION

In general, this systematic review provides some evidence that exercise therapy is effective to induce an immunomodulatory response in KOA patients characterized by mainly increased anti-inflammatory signaling and decreased pro-inflammatory signaling. Evidence for both acute and basal exercise effects was found, however it seemed that this effect was more pronounced in trained versus untrained status. Both intra-articular and systemic inflammation was downregulated by exercise therapy. Furthermore, exercise therapy can be considered as safe for the KOA population because no exacerbation of inflammation was observed.

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The authors declare no conflicts of interest.

SUPPLEMENTARY TABLES

Supplementary material (supplementary tables 1 and 2) can be obtained upon request by contacting the corresponding author via ivan.bautmans@vub.be.

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Table 1:
Search terms PICO

	P (Osteoarthritis)	I (Physical Exercise)	C	O (inflammatory markers & BDNF)
PubMed	"Osteoarthritis"[Mesh] osteoarthr*	"Exercise"[Mesh] "Exercise Movement Techniques"[Mesh] "Exercise Therapy"[Mesh] "Resistance Training"[Mesh] "Sports"[Mesh] strength training aerobic training exercise		"Inflammation"[Mesh] "Acute-Phase Proteins"[Mesh] "Interleukins"[Mesh] "Cytokines"[Mesh] "Brain-Derived Neurotrophic Factor"[Mesh] inflammation acute phase reaction interleukin cytokine brain derived neurotrophic factor BDNF
WOS	osteoarthritis osteoarthr*	Exercise Exercise Movement Techniques Exercise Therapy Resistance Training Sports strength training aerobic training		Brain-Derived Neurotrophic Factor Inflammation Acute-Phase Proteins Interleukins Cytokines BDNF brain derived neurotrophic factor inflammation acute phase reaction interleukin cytokine
PEDro	osteoarthr*	exercis* physical* training therapy		inflam* tumor necrosis factor* cytokin* interleukin* "brain derived neurotrophic factor" BDNF

No filters were used in PubMed. In Web of Science 'Basic Search/Topic/All years' was chosen. In PEDro we chose for 'Method: Clinical Trial' on the 'Advanced Search' page. Only search terms in the 'Abstract & Title' box were filled in and matched with 'AND'.

Table 2:
Exercise protocols

Reference	Inter-vention Period	Frequency	Duration	Intensity/load	Exercise modality
Aerobic (A) & Strength (S)					
Beavers et al., 2014[1]	18 mo	3x/wk	1h 2x 15' 20' 10'	 50-75% HRR 1-2x10-12 reps; 1'-1.5' rest interval NA	exercise: A: walking (before and after strength) S: leg ext., leg pr., seated: leg curl & calf raise, row, vertical chest or incline press cool down
Loeser et al., 2017	18mo	3x/wk	1h 2x 15' 20' 10'	 50-75% HRR 1-2x10-12 reps; 1'-1.5' rest interval NA	Exercise: A: walking (before and after strength) S: leg ext., leg pr., seated: leg curl & calf raise, row, vertical chest or incline press Cool down
Messier et al., 2000	6 mo	3x/wk	1h 5' 2x 10' 20'-30' 5''	 N/A 50-75% HRR 10-12 reps; 1'-1.5' rest interval NA	 warm up exercise: A: walking (before and after strength) S: leg ext., toe raise, leg curl, military press, upright row, chest fly, pelvic tilt (upper body: dumbbells; lower body: cuff weights) cool down
Messier et al., 2013[1]	18 mo	3x/wk	1h 2x 15' 20' 10'	 50-75% HRR 1-2x10-12 reps; 1'-1.5' rest interval NA	 exercise: A: walking (before and after strength) S: leg ext., leg pr., seated: leg curl & calf raise, row, vertical chest or incline press cool down
Miller et al., 2004	18mo	3x/wk	1h 2x15' 15' 15'	 50-75% HRR 2x12 reps; 1'-1.5' rest interval (Weight progressive ↑) NA	 Exercise: A: walking (before and after strength) S: leg extension, leg curl, heel raise, step-up (ankle cuff weights, weighted vest) Cool down
Nicklas et al., 2004[2]	18 mo	3x/wk	1h 2x 15' 15' 15'	 50-75% HRR 2x12 reps; 1'-1.5' rest interval (Weight progressive ↑) NA	 exercise: A: walking S: leg extension, leg curl, heel raise, step-up (ankle cuff weights, weighted vest) cool down
Other					
Aguiar et al., 2014	12 wks	3x/wk	80' 3x30"	 NA	exercise: F: passive stretching: iliopsoas, rectus femoris, iliotibial band active stretching: Hamstrings, Gastrocnemius, hip adductors S: Hamstrings, Quadriceps, Glut max, Glut med, Abdominals
			NA	3x10 reps (wk 1-2), 3x15 reps (wk 3-4); 60% MF 3x10 reps (wk 5-6), 3x15 reps (wk 7-8); 70% MF 3x10 reps (wk 9-10), 3x15 reps (wk 11-12); 80% MF 60" rest interval	Open kinematic chain (OKC)-exercises with leg weights
			NA	same progression in sets and reps as OKC-ex same progression in sets and reps as OKC-ex	squat exercises (45° knee flexion) Abdominals: isometric (wk 1-4); concentric (wk 5-12)
Liu et al., 2019	12wks	5x/wk	1h 10' 30' 10' 10'	 70-75% HRmax 	Exercises: Warm-up Cycling (A) or Tai Chi or Baduanjin Breathing techniques Relaxation
Aerobic (A)					
Alkatan et al., 2016	12wks	3x/wk	Progressive ↑ From 20-30' to 40-50'	Progressive ↑ 40-50% HRR 60-70% HRR	Exercises: cycling (A) or swimming (A)
Gomes et al., 2012	12 wks	3x/wk	Progressive ↑ 5' 30' (+5' /2 wks) 5'	Progressive ↑ NA HRmax: 70% (wk 1-3); 75% (wk 4-7); 80% (wk 8-12) NA	TRAINING (T) warm up: walking exercise: walking cool down: walking
		1x Pre T, 1xPost T	50' 2' 18' 30'	 1mph (0% inclination) 2 mph (0% incliniation)	ACUTE BOUT warm up: walking treadmill exercise: walking treadmill cool down: lie down
Gomes et al., 2014	12 wks	3x/wk	Progressive ↑ 5' 30' (+5' /2 wks) 5'	Progressive ↑ NA HRmax: 70% (wk 1-3); 75% (wk 4-7); 80% (wk 8-12) NA	TRAINING(T) warm up: walking (land + aquatic) exercise: walking (land + aquatic) cool down: walking (land + aquatic)
		1x Pre T, 1xPost T	50' 2' 18' 30'	 1mph (0% inclination) 2 mph (0% incliniation)	ACUTE BOUT warm up: walking treadmill exercise: walking treadmill cool down: lie down
Jayabalan et al., 2019	45' x2	1x pre T, after 15', 30' and 45'	45'	40-60% HRR 1.3m/s	ACUTE BOUT 1x45': continuous walking on treadmill 1x45': interval walking on treadmill
Samut et al., 2015	6 wks	3x/wk	Progressive ↑ 5' wk1: 15' (+5'/wk)[3] 5'	Progressive ↑ <60% HRmax[3][4] HRmax[4]: 65-70% (wk 1-4); 70-75% (wk 5-6) NA	warm-up: walking on treadmill exercise: walking on treadmill cool down: walking on treadmill[3]
Strength (S)					
Armagan et al., 2012	6mo	1x/day	NA	2 sets of 20 reps; 2' break between the sets	Quadriceps isometric and isotonic strengthening, joint range of motion, and hamstring stretching exercises

Germanou et al., 2013	24h		NA		
		1xPre,1x post, 1x24hpost		1x5 reps; maximal; (30" rest interval); at random: 90°/s, 120°/s, 150°/s	test: isokinetic (dynamometry chair): concentric knee ext/flex; affected leg:
		1x		6x10 reps; maximal; (30" rest interval); 90°/s (set 1+6); 120°/s (set 2+5); 150°/s (set 3+4)	exercise: isokinetic (dynamometry chair): concentric knee ext/flex; affected leg
Helmark et al., 2010	**1 day**	**1 session**	**±1h**		
			5'	NA	warm up: bicycle
			NA		5-7 RM test: leg press; one leg (start at 90° knee flex)
			NA	25x10 reps; 1.5' rest interval; 60% 1RM [5]	exercise:leg press; one leg (start at 90° knee flex)
Helmark et al., 2012	**1 day**	**1 session**			
			5'	NA	exercise: warm up: bicycle
			NA	10x8 reps; 2.5' rest interval; 60% 1RM [6]	leg press (knee extension); one leg (start at 90° knee flex)
Kim et al., 2021	**4-6wks**	**3x/wk**	**60'**	**Progressive ↑**(with ankle cuffs)	Aquatic exercises
			10'		Warm-up
			20'		Flexibility and strength
			20'		Low intensity endurance: e.g. walking
			10'		Cool down
Samut et al., 2015	**6 wks**	**3x/wk**	NA	**Progressive ↑**	
			5'	<60% HRmax[3][4]	warm-up: walking on treadmill
			NA	session 1: 1x5 reps (60°/s, 90°/s, 120°/s, 180°/s), + 1 set each following session until 6 sets (20" rest interval between sets, 2' rest between legs)	exercise: isokinetic (dynamometry chair): concentric knee ext/flex, both legs (ROM knee adjusted between 10°-80°)
			5'	NA	cool down: walking on treadmill[3]
Simão et al., 2012	**12 wks**	**3x/wk**			
					SQUAT-group:
			10'	HRmax: 70%	warm up: bicycle
			NA	3" 10° knee flex /3" 60° knee flex progressive ↑ volume (time↑/ # sets↑, rest time↓)	exercise: SQUAT (isometric) start 10° knee flex/end 60° knee flex
					PLATFORM-group
			10'	HRmax: 70%	warm up: bicycle
			NA	3" 10° knee flex /3" 60° knee flex progressive ↑ volume (time↑/ # sets↑, rest time↓, vibratory frequency 35-40Hz; amplitude 4; acceleration 2.78-3.26g)	exercise: SQUAT on WHOLE BODY VIBRATION PLATFORM (isometric): start 10° knee flex/end 60° knee flex
Simão et al., 2019	**12wks**	**3x/wk**	10'	HRmax: 70%	SQUAT-group:
			NA	3" 10° knee flex /3" 60° knee flex progressive ↑ volume (time↑/ # sets↑, rest time↓)	warm up: bicycle exercise: SQUAT (isometric) start 10° knee flex/end 60° knee flex
Zhao et al., 2019	**2y**	**2x/day**	30'	NA	Exercise: static low angle squat

[1]months 1-6: center based, months 7-18: center+home or home based, home based: Thera-Band exercise program; [2]months 1-4: center based; months 5-18: center, home or center+home based; [3]Information obtained from author; [4]age related heart rate according Karvonen formula; [5]1RM determined by 5-7 RM test; [6]1RM determined by 5 RM test (3 months before intervention); **h**=hour; **MF**= maximal force (determined by 10 RM-test); **mo**=month; **mph**=mile per hour; **NA**= not available; **OKC-ex**= Open kinematic chain exercises, **reps**=repetitions; **s**=seconds; **T**= training; **wk**=week; **HRR**= heart rate reserve; **HRmax**= maximum heart rate; **y**= year

Table 3:
Detailed individual results

Reference Study design	Population Sample size: N (% retention) Female: % (% retention) Male: % (% retention) Age (years)	Number of study arms / (G) Interventions for each arm	Interv ention period	Sampling	Results	Effect size
CRP Basal effects						
Nicklas et al., 2004 RCT	OAk (Rx) N=316 (79.7%) Female[16]: 71.7% (NA)[1] Male[16]: 28.9% (NA)[1] (G1) 69 ± 6[6] (G2) 68 ± 7[6] (G3) 68 ± 5[6] (G4) 69 ± 6[6]	4/ (G1) Aerobic + strength (G2) Diet + aerobic + strength (G3) Diet (G4) Control: healthy lifestyle 1h, 3x/week: Aerobic (walking 15 min, 50-75%HRR), resistance training (2x12 repetitions, 15 min), aerobic (15 min), cooling down (15 min)	18mo	Serum (T0) at baseline (T1) at 6 mo (T2) at 18 mo	**Between group differences: G1 vs G2 vs G3 vs G4** T0: ND: 6.8 ± 7.8 vs 6.5 ± 7.9 vs 6.0 ± 6.5 vs 5.9 ± 6.0 µg/mL[2] ΔCRP T0 - T1: -0.07 ± 0.42 vs -0.11 ± 0.52 vs -0.11 ± 0.72 vs 0.08 ± 0.63 µg/mL[2] T0 - T2: -0.02 ± 0.47 vs -0.18 ± 0.54 vs-0.13 ± 0.53 vs 0.35 ± 1.9 µg/mL[2] G1: ND (p=0.57) G3↓ vs (G1 + G4) (Δ log CRP: -0.26 ± (0.07) vs 0.04 ± (0.07) µg/mL (p=0.01))	
Samut et al., 2015 RCT	OAk (Sx+Rx) N=42 (95%) Female: 90% (NA) Male: 10% (NA) (G1) 62.46± 7.71[6] (G2) 57.57 ± 5.79[6] (G3) 60.92 ± 8.85[6]	3/ (G1) Strength (isokinetic) (G2) Aerobic (G3) Info disease & recommendations about precautions Strength: 3x/week 5 min warm-up on treadmill 5 concentric flexion and extension exercises Aerobic: 3x/week 5 min warm-up on treadmill Week 1-4: 65-70% of age related HR Week 5-6: 70-75% of age related HR 5 min cool-down period	6wks	Serum (T0) at baseline (T1) at 6 wks	**Between group differences: G1 vs G2 vs G3** T0: ND: 0.302 (0.100 - 1.600) vs 0.488 (0.105 - 1.450) vs 0.386 (0.159 - 1.170) mg/L (p=0.460) T1: 0.229 (0.100 - 0.785) vs 0.432 (0.127 - 0.875) vs 0.381 (0.100 - 1.190) mg/L[2] **Within group differences: T0 vs T1** G1: ND: (0.302 (0.100 - 1.600) vs 0.229 (0.100 - 0.785) mg/L (p= 0.087) G2: ND: (0.488 (0.105 - 1.450) vs 0.432 (0.127 - 0.875) mg/L (p= 0.072) G3: ND: (0.386 (0.159 - 1.170) vs 0.381 (0.100 - 1.190) mg/L (p= 0.382)	
hsCRP	OAk (NA)	2/	4-6 wks	Blood	**Between group differences: G1 vs G2**	i.d.a.
Kim et al., 2021 RCT	N= 43 (98%) Female: 19 (44%) Male: 24 (56%) (G1) 67.4 ± 6.0 (G2) 66.9 ± 6.3	(G1) Strength (i.e. aquatic exercises) (G2) Control: usual care + brochure on perioperative nutrition 3x/week 60 min until scheduled total knee arthroplasty surgery (4-8 weeks) 10 min warm-up, 20 min flexibility and strength, 20 min low intensity endurance, 10 min cool down. Resistance equipment (i.e. ankle cuffs) was added depending on the tolerance to increase intensity		(T0) at baseline (T1) 1 week before TKA surgery	T0: ND: 5.72 ± 7.48 vs 2.37 ± 3.57 [2] **SMD:** Log(hsCRP): -0.25 [95% CI: -0.69 to 0.20] (p=0.27)	
Germanou et al., 2013 NRUCT	(G1) OAk (Sx+Rx) N=10 (100%) Female[16] :100% (100%) 58.9 ± 5.9 yrs (G2) Healthy Knees N=10 (100%) Female[16]: 100% (100%) 62.4 ± 5.1	2/ Strength (i.e. isokinetic exercises) 6 sets of 10 maximal concentric knee extensions/flexions	24h	Serum (T0) at baseline (T1) 24h post-exercise	**Between group differences: G1 vs G2** T0: G1↑ vs G2[4] (p=0.001) T1: G1↑ vs G2[4] (p=0.001) **Within group differences: T0 vs T1** G1: ND[2][4]; 4.54 ± (0.39) vs 4.60 ± (0.58) mg/L[3] G2: ND[2][4]; 2.67 ± (0.23) vs 2.73 ± (0.39) mg/L[5]	ES_{G1}= 0.039
Beavers et al. 2014 RCT	OAk (Sx+Rx) N=454 (78%) Female [16]: 71.3% (NA)[1] Male[16]: 28.7% (NA)[1] 65.6 ± 6.2	3/ (G1) Aerobic + strength (G2) Diet + aerobic + strength (G3) Diet 1h 3x/week: Aerobic (walking 15 min), strength training (20 min), aerobic phase (15 min), cooling down (10 min)	18mo	Plasma (T0) at baseline (T1) at 6 mo (T2) at 18 mo (at least 24h after last acute bout of exercise)	**Within group differences: T0 vs T1** G1: ND: (0.302 (0.100 - 1.600) vs 0.229 (0.100 - 0.785) mg/L (p= 0.087) G2: ND: (0.488 (0.105 - 1.450) vs 0.432 (0.127 - 0.875) mg/L (p= 0.072) G3: ND: (0.386 (0.159 - 1.170) vs 0.381 (0.100 - 1.190) mg/L (p= 0.382)	
CRP Acute effects						
Germanou et al., 2013	(G1) OAk (Sx+Rx) N=10 (100%) Female[16]: 100% (100%)	2/ Strength (i.e. isokinetic exercises)	<24 h (1 x)	Serum	**Between group differences: G1 vs G2** T0: G1↑ vs G2[4] (p=0.001)	

	(G2) Healthy Knees N=10 (100%) Female[16]: 100% (100%) 62.4 ± 5.1	6 sets of 10 maximal concentric knee extensions/flexions		(T1) immediately post-exercise	**Within group differences: T0 vs T1** G1: ND[2][4]; 4.54 ± (0.39) vs 4.63 ± (0.70) mg/L[5] G2: ND[2][4]; 2.67 ± (0.23) vs 2.76 ± (0.51) mg/L[5]	ES_{G1}= 0.052
CRPM Basal effects						
Loeser et al., 2017 RCT	OAk (Sx+Rx) N= 429 (94.49%) Female [16]: 100% (NA) 65.76 ± 6.22 yrs	3/ (G1) Aerobic + strength (G2) Diet + aerobic + strength (G3) Diet 1h, 3x/week: Aerobic (walking 15 min), strength training (20 min), aerobic phase (15 min), cooling down (10 min)	18mo	Serum (T0) at baseline (T1) at 6 mo (T2) at 18 mo (at least 24h after last acute bout of exercise)	Secondary analysis data IDEA- trial **Between group differences: G1 vs G2 vs G3: ND[2]** $T2^{(20)}$-T0: log(CRPM)= -0.08 ± (0.03) (-0.84%) vs -0.11 ± (0.03) (-1.12%) vs -0.08 ± (0.03) (-0.83%)	
					Within group differences: T0 vs T1 vs T2: ND[2][4]	
IL-1β Basal effects						
Alkatan et al., 2016 RCT	OAk (Sx + Rx) N= 48 (83.33%)[17] Female: 91.67% (NA)[1] Male: 8.33% (NA)[1] (G1) 61 ± 1 (G2) 59 ± 2	2/ (G1): Aerobic (i.e. cycling) (G2): Aerobic (i.e. swimming) From 20-30 min 3x/week at 40-50% HRR to 40-50min 3x/week at 60-70% HRR	12 wks	Plasma (T0) at baseline (T1) at week 12 (at least 48h after last exercise session)	**Within group differences: T0 vs T1** G1: ND: 57 ± (9) vs 48 ± (8) pg/mL[2] G2: ND: 38 ± (5) vs 37 ± (4) pg/mL[2]	ES_{G1}= -0.237 ES_{G2}= -0.050
Zhao et al., 2019 RCT	OAk (Sx) N= 55 Female: [4] Male: [4] (54-65)	2/ (G1): Strength (i.e. static low angle squat) (G2): Control (NA) 2x/day 30 min static low angle squats	2 y	Synovial fluid (OA knee) (T0) at baseline (T1) after 12 months	**Within group differences: T0 vs T1** G1: ↓: 80.23 ± 6.54 vs 43.75 ± 5.23 pg/mL (p<0.001)	ES_{G1}= -6.199
Armagan et al., 2012 RCT	OAk (Sx+Rx) N= 55 (90.91%) Female: 100% (NA)[1] (G1) 63.30 ± 6.45 (G2) 59.85 ± 6.67	2/ (G1) Strength + calcitonin treatment (G2) Strength 1x/day 2 sets of 20 repetitions with 2 min rest between each set at home	6 mo	Serum (T0) at baseline (T1) at 6 months	**Between group differences: G1 vs G2** T0: ND: 2.36 (2.00-2.85) vs 2.06 (1.84-2.39) (p= 0.354) T1: ND: 2.16 (2.02 -2.72) vs 2.19 (1.60-3.00) (p=0.205)	ES_{T1}= 0,049
					Within group differences: T0 vs T1 G1: ND: 2.36 (2.00-2.85) vs 2.16 (2.02 - 2.72) (p=0.301) G2: ND: 2.06 (1.84 -2.39) vs 2.19 (1.60-3.00) (p=0.235)	ES_{G2}= 0.146
Messier et al., 2000 RCT	OAk (Rx) N=24 (87.5%) Female[16]: 70.8% (NA) Male[16]: 29.2% (NA) (G1) 69 ± 5[6] (G2) 67 ± 4[6]	2/ (G1) Aerobic + strength (G2) Diet+ aerobic+ strength 3x/week, 1h/day 5 min warm-up, 10 min walking (50-75% HRR), 20-30 min strength training, 10 min walking (50-75% HRR), 5 min cool-down	6 mo	Synovial fluid (most symptomatic knee joint) (T0) at baseline (T1) at 6 mo	**Combined data for two intervention groups ("within group"):** T0 vs T1↓ ; 25.325 ± (9.75) vs 8.306 ± (6.112) pg/mL (p<0.04)[12]	
IL-2 Basal effects						
Alkatan et al., 2016 RCT	OAk (Sx + Rx) N= 48 (83.33%)[17] Female: 91.67% (NA)[1] Male: 8.33% (NA)[1] (G1) 61 ± 1 (G2) 59 ± 2	2/ (G1): Aerobic (i.e. cycling) (G2): Aerobic (i.e. swimming) From 20-30 min 3x/week at 40-50% HRR to 40-50min 3x/week at 60-70% HRR	12 wks	Plasma (T0) at baseline (T1) at week 12 (at least 48h after last exercise session)	**Within group differences: T0 vs T1** G1: ND: 56 ± (10) vs 71 ± (10) pg/mL[2] G2: ND: 49 ± (9) vs 41 ± (8) pg/mL[2]	ES_{G1}= 0.335 ES_{G2}= -0.175
IL-4 Basal effects						
Alkatan et al., 2016 RCT	OAk (Sx + Rx) N= 48 (83.33%)[17] Female: 91.67% (NA)[1] Male: 8.33% (NA)[1] (G1) 61 ± 1 (G2) 59 ± 2	2/ (G1): Aerobic (i.e. cycling) (G2): Aerobic (i.e. swimming) From 20-30 min 3x/week at 40-50% HRR to 40-50min 3x/week at 60-70% HRR	12 wks	Plasma (T0) at baseline (T1) at week 12 (at least 48h after last exercise session)	**Within group differences: T0 vs T1** G1: ND: 45 ± (7) vs 38 ± (4) pg/mL[2] G2: ND: 45 ± (7) vs 36 ± (4) pg/mL[2]	ES_{G1}= -0.285 ES_{G2}= -0.366
IL-5 Basal effects						
Alkatan et al., 2016 RCT	OAk (Sx + Rx) N= 48 (83.33%)[17] Female: 91.67% (NA) [1] Male: 8.33% (NA)[1] (G1) 61 ± 1 (G2) 59 ± 2	2/ (G1) Aerobic (i.e. cycling) (G2) Aerobic (i.e. swimming) From 20-30 min 3x/week at 40-50% HRR to 40-50min 3x/week at 60-70% HRR	12 wks	Plasma (T0) at baseline (T1) at week 12 (at least 48h after last exercise session)	**Within group differences: T0 vs T1** G1: ND: 62 ± (5) vs 57 ± (6) pg/mL[2] G2: ND: 39 ± (3) vs 39 ± (4) pg/mL[2]	ES_{G1}= -0.203 ES_{G2}= -0.000
IL-6 Basal effects						
Nicklas et al., 2004 RCT	OAk (Rx) N=316 (79.7%) Female[16]: 71.7% (NA)[1] Male[16]: 28.9% (NA)[1] (G1) 69 ± 6[6] (G2) 68 ± 7[6]	4/ (G1) Aerobic + strength (G2) Diet + aerobic + strength (G3) Diet (G4) Control: healthy lifestyle	18 mo	Serum (T0) at baseline (T1) at 6 mo (T2) at 18 mo	**Between group differences: G1 vs G2 vs G3 vs G4** T0: ND: 4.4 ± 3.1 vs 4.9 ± 3.0 vs 4.7 ± 3.4 vs 4.7 ± 3.2 pg/mL[2] Δ IL-6 T0 - T1: 0.15 ± 1.8 vs -0.35 ± 2.15 vs -	

	(G3) 68 ± 5[6] (G4) 69 ± 6[6]	1h 3x/week: Aerobic (walking 15 min, 50-75%HRR), resistance training (2x12 repetitions, 15 min), aerobic (15 min), cooling down (15 min)			0.51 ± 2.1 vs 0.19 ± 2.8 pg/mL[2] T0 - T2: 0.02 ± 2.4 vs -0.35 ± 1.8 vs -0.71 ± 2.4 vs 0.27 ± 2.8 pg/mL[2] G1: ND (p=0.86) G3 ↓ (p=0.009) G3 ↓ vs (G1 + G4) (Δ log IL-6: -0.13 ± (0.04) vs -0.01 ± (0.04) pg/mL)[2]	
Samut et al., 2015 RCT	OAk (Sx+Rx) N=42 (95%) Female:90% (NA) Male: 10% (NA) (G1) 62.46± 7.71[4] (G2) 57.57 ± 5.79[6] (G3) 60.92 ± 8.85[6]	3/ (G1) Strength (isokinetic) (G2) Aerobic (G3) Control: info disease & recommendations about precautions Strength: 3x/week 5 min warm-up on treadmill 5 concentric flexion and extension exercises Aerobic: 3x/week 5 min warm-up on treadmill Week 1-4: 65-70% of age related HR Week 5-6: 70-75% of age related HR 5 min cool-down period	6 wks	Serum (T0) at baseline (T1) at 6 wks	**Between group differences: G1 vs G2 vs G3** T0: 0.732 (0.037 - 2.497) vs 0.861 (0.173 - 1.894) vs 0.259 (0.037 - 1.507) pg/mL (p=0.113) T1: 0.947 (0.037 - 6.044) vs 0.625 (0.037 - 2.024) vs 0.381 (0.037 - 1.259) pg/mL[2] **Within goup differences: T0 vs T1** G1: ND: (0.732 (0.037 - 2.497) vs 0.947 (0.037 - 6.044) pg/mL, (p=0.753) G2: ND: (0.861 (0.173 - 1.894) vs 0.625 (0.037 - 2.024) pg/mL, (p= 0.706) G3: ND: (0.259 (0.037 - 1.507) vs 0.381 (0.037 - 1.259) pg/mL, (p=0.583)	
Germanou et al., 2013 NRCT	(G1) OAk (Sx+Rx) N=10 (100%) Female[16]: 100% (100%) 58.9 ± 5.9 (G2) Healthy Knees N=10 (100%) Female[16]: 100% (100%) 62.4 ± 5.1	2/ Strength (i.e. isokinetic exercises) 6 sets of 10 maximal concentric knee extensions/flexions	24h	Serum (T0) at baseline (T1) 24 h post-exercise	**Between group differences: G1 vs G2** T0: G1↑ vs G2[4] (p=0.04) T1: G1↑ vs G2[4][8] (p=0.04) **Within group differences T0 vs T1:** G1: ND[2][4][8] ; 4.85 ± (0.70) vs 4.92 ± (0.58) pg/mL[5] G2: ND[2][4][8] ; 3.87 ± (0.42) vs 3.94 ± (0.49) pg/mL[5]	ES_{G1}= 0.035
Gomes et al., 2012 NRUCT	OAk (Sx+Rx) N=15 (100%) Female: 100% (100%) 67 ± 4	1/ Aerobic (walking) 3x/week 5min warm up walking from 30 min (week 1) to 55 min (week 12) from 70% HRmax to 80% HRmax 5 min cool-down	12 wks	Plasma In the morning (T0) at baseline (T1) at 12 wks	**Within group differences:** T0 vs T1: ND; 1.7 ± 2.8 [-0.02 to 3.5] vs 1.2 ± 0.8 [0.8 to 1.7] pg/mL (p>0.05) Cohen's d=0.343	ES_{G1}= -0.278
Alkatan et al., 2016 RCT	OAk (Sx + Rx) N= 48 (83.33%)[19] Female: 91.67% (NA) [1] Male: 8.33% (NA)[1] (G1) 61 ± 1 (G2) 59 ± 2	2/ (G1) Aerobic (i.e.cycling) (G2) Aerobic (i.e. swimming) From 20-30 min 3x/week at 40-50% HRR to 40-50min 3x/week at 60-70% HRR	12 wks	Plasma (T0) at baseline (T1) at week 12 (at least 48h after last exercise session)	**Within group differences: T0 vs T1** G1 ↓: 90 ± (10) vs 79 ± (9) pg/mL, (p<0.05) G2 ↓: 100 ± (11) vs 76 ± (7) pg/mL, (p<0.05)	ES_{G1}= -0.259 ES_{G2}= -0.596
Aguilar et al., 2014 NRUCT	OAk (Sx+Rx) N=27 (81.5%) Female: NA (81.8%) Male: NA (18.2%) 58.8 ± 6.4	1/ Other: Flexibility + strength 3x/week, 80min/day Supervised Week 1-4: 60% of max. load Week 5-8: 70% max. load Week 9-12: full weight determined by 10-MR test	12 wks	Serum (T0) at baseline (T1) at 12 wks (change after training)	**Within group differences:** T0 vs T1↓[4] (p<0.001); 1.96 (17) vs 1.37 [0.09 - 3.77] pg/mL[5][12]	ES_{T1-T0}= -0.513
Kim et al., 2021 RCT	OAk (NA) N= 43 (98%) Female: 19 (44%) Male: 24 (56%) (G1) 67.4 ± 6.0 (G2) 66.9 ± 6.3	2/ (G1) Strength (i.e. aquatic exercises) (G2) Control: usual care + brochure on perioperative nutrition 3x/week 60 min until scheduled total knee arthroplasty surgery (4-8 weeks) 10 min warm-up, 20 min flexibility and strength, 20 min low intensity endurance, 10 min cool down. Resistance equipment (i.e. ankle cuffs) was added depending on the tolerance to increase intensity	4-6 wks	Blood (T0) at baseline (T1) 1 week before TKA surgery	**Between group differences: G1 vs G2** T0: ND: 4.16 ± 4.04 vs 2.68 ± 2.46 [2] **SMD:** Log (IL-6); -0.02 [95% CI: -0.32 to 0.28] (p=0.90)	i.d.a.
Messier et al., 2013 RCT	OAk (Sx+Rx) N=454 (88%)[17] Female: 71.6% (NA) Male: 28.4% (NA) 66.6 ± 6	3/ (G1) Aerobic + strength (G2) Diet + aerobic + strength (G3) Diet 1h/day, 3x/week: 15 min walking 20 min strength training 15 min aerobic exercises 10 min cool-down	18 mo	Plasma (T0) at baseline (T1) at 6 mo (T2) at 18 mo (at least 24h after last acute bout of exercise)	N=Results for Multiple imputation-based Model (50 Multiply imputed data sets) **Between group differences: G1 vs G2 vs G3 (p=0.008)** T0: 3.0 [2.6 to 3.3] vs 3.2 [2.9 to 3.6] vs 3.2 [2.8 to 3.6] pg/mL T1: 2.9 [2.6 to 3.3] vs 2.8 [2.5 to 3.2] vs 2.7 [2.4 to 3.1] pg/mL T2: 3.0 [2.7 to 3.3] vs 2.7 [2.4 to 3.1] vs 2.7 [2.3 to 3.0] pg/mL T2[9]: 3.1 [2.9 to 3.4] vs 2.7 [2.5 to 3.0]	

					vs 2.7 [2.4 to 3.0] pg/mL **Pairwise between-groups differences at T2[9][10]** G1 vs G2: 0.39[-0.03 to 0.81] pg/mL (p=0.007) G1 vs G3: 0.43[0.01 to 0.85] pg/mL (p=0.006) G3 vs G2: -0.04[-0.47 to 0.40] pg/mL (p=0.98) **Within group differences: ΔT0 vs T2** G1: 0.1 pg/mL: no reduction (0%) G2: -0.5 pg/mL: reduction of 15% G3: -0.5 pg/mL: reduction of 16%	
IL-6 Acute effects						
Germanou et al., 2013 NRCT	(G1) OAk (Sx+Rx) N=10 (100%) Female[16]: 100% (100%) 58.9 ± 5.9 (G2) Healthy Knees N=10 (100%) Female[16]: 100% (100%) 62.4 ± 5.1	2/ Strength (i.e. isokinetic exercises) 6 sets of 10 maximal concentric knee extensions/flexions	<24 h (1 x)	Serum (T0) at baseline (T1) immediately post-exercise	**Between group differences: G1 vs G2** T0: G1↑ vs G2[4] (p=0.04) T1: G1↑ vs G2[2][4][8] **Within group differences T0 vs T1:** G1↑[4] (p=0.04); 4.85 ± (0.70) vs 5.50 ± (0.61) pg/mL[5] G2: ND[2][4][8]; 3.87 ± (0.42) vs 4.01 ± (0.70) pg/mL[5]	ES_{G1}= 0.314
Helmark et al., 2010 RCT	OAk (Sx+Rx) N=31 (93.5%) Female: 100% (100%) (G1) 66 ±6 (G2) 67 ± 7	2/ (G1) Strength (G2) Control: non exercise 5 min warm-up on bicycle 25 sets of 10 repetitions at 60% of 1RM on legg-press machine	<24h (1x)	Synovial fluid: - peri-synovial - intra-articular at latest 1h after exercise; 6 samples every 30' (3h): samples were later pooled: T1= sample 1 to 3 (30', 60', 90'); T2 = sample 4 to 6 (120', 150', 180')	**Between group differences:** T1: G1 vs G2: ND [2][4] **Within group differences T1 vs T2:** Intra-articular: G1↑ (p<0.05)[4][8] G2↑ (p<0.05)[4][8] peri-synovial: G1↑ (p<0.05)[4][8] G2↑ (p<0.05)[4][8]	
Helmark et al., 2012 NRUCT	OAk (Sx+Rx) N=11 (63.6%) Female: NA Male: NA 61 ± 11	1/ Strength 30min one-legged extension at 60% 1RM, 10 sets of 8 repetitions	<24h (1x)	Synovial fluid (knee with most severe symptoms) (T0) at baseline (± 3 mo before exercise intervention) (T1) 15-30' after exercise	**Within group differences T0 vs T1:** ND [2][4][18]; 97.96 (22.53-253.27) vs 149.66 (23.20 -612.26) pg/mL[5]	ES_{T1-T0}: 0.9
Gomes et al., 2012 NRUCT	OAk (Sx+Rx) N=15 (100%) Female: 100% (100%) 67 ± 4	1/ Aerobic 3x/week 5min warm up walking from 30 min (week 1) to 55 min (week 12) from 70% HRmax to 80% HRmax 5 min cool-down	12 wks	Plasma (T0) at baseline: (T00) pre-acute EX (T01) post-acute EX (T02) 30' post-acute EX (T1) at 12 wks: (T10) pre-acute EX (T11) post-acute EX (T12) 30' post-acute EX	**Within group differences:** i.d.a. ND [2][4][13][18]	
IL-6sR Basal effects						
Nicklas et al., 2004 RCT	OAk (Rx) N=316 (79.7%) Female[16]: 71.7% (NA)[1] Male[16]: 28.9% (NA)[1] (G1) 69 ± 6 (G2) 68 ± 7 (G3) 68 ± 5 (G4) 69 ± 6	4/ (G1) Aerobic + strength (G2) Diet + aerobic + strength (G3) Diet (G4) Control: healthy lifestyle 1h 3x/week: Aerobic (walking 15 min, 50-75%HRR), resistance training (2x12 repetitions, 15 min), aerobic (15 min), cooling down (15 min)	18 mo	Serum (T0) at baseline (T1) at 6 mo (T2) at 18 mo	**Between group differences: G1 vs G2 vs G3 vs G4** T0: ND: 34581 ± 9596 vs 35156 ± 11075 vs 35197 ± 8911 vs 38040 ± 10764 pg/mL[2] Δ IL-6sR T0 - T1: -856 ± 5135 vs -759 ± 3235 vs -838 ± 3645 vs -1247 ± 5159 pg/mL[2] T0 - T2: 94 ± 4399 vs -746 ± 4166 vs -1522 ± 8344 vs -739 ± 4910 pg/mL[2] G1: ND (p=0.44) G3: ND (p=0.81) IL-6/IL-6sR G1: ND[2] G3: ND[2]	
IL-7 Basal effects						
Alkatan et al., 2016 RCT	OAk (Sx + Rx) N= 48 (83.33%)[17] Female: 91.67% (NA) [1] Male: 8.33% (NA)[1] (G1) 61 ± 1 (G2) 59 ± 2	2/ (G1) Aerobic (i.e. cycling) (G2) Aerobic (i.e. swimming) From 20-30 min 3x/week at 40-50% HRR to 40-50min 3x/week at 60-70% HRR	12 wks	Plasma (T0) at baseline (T1) at week 12 (at least 48h after last exercise session)	**Within group differences: T0 vs T1** G1: ND: 53±(6) vs 48±(6) pg/mL[2] G2: ND: 45±(5) vs 42 ± (6) pg/mL[2]	ES_{G1}= -0.186 ES_{G2}= -0.122
IL-8 Basal effects						
Alkatan et al., 2016	OAk (Sx + Rx) N= 48 (83.33%)[17]	2/ (G1) Aerobic (i.e. cycling)	12 wks	Plasma	**Within group differences: T0 vs T1** G1: ND: 260 ± (34) vs 275 ± (35) pg/mL[2]	ES_{G1}= 0.097

RCT	Female: 91.67% (NA)[1] Male: 8.33% (NA)[1] (G1) 61 ± 1 (G2) 59 ± 2	(G2) Aerobic (i.e. swimming) From 20-30 min 3x/week at 40-50% HRR to 40-50min 3x/week at 60-70% HRR		(T0) at baseline (T1) at week 12 (at least 48h after last exercise session)	G2: ND: 225 ± (22) vs 241 ± (14) pg/mL[2]	ES_{G2}= 0.199
IL-8 Acute effects						
Helmark et al., 2010 RCT	OAk (Sx+Rx) N=31 (93.5%) Female: 100% (100%) (G1) 66 ±6 (G2) 67 ± 7	2/ (G1) Strength (G2) Control: non exercise 5 min warm-up on bicycle 25 sets of 10 repetitions at 60% of 1RM on legg-press machine	<24h (1x)	Synovial fluid: - peri-synovial - intra-articular At latest 1h after exercise; 6 samples every 30' (3h); samples were later pooled: T1= sample 1 to 3 (30', 60', 90') T2 = sample 4 to 6 (120', 150', 180')	**Between group differences G1 vs G2:** T1: ND[2][4] **Within group differences T1 vs T2:** Intra-articular: G1↑ ($p<0.05$)[4][8] G2↑ ($p<0.05$)[4][8] peri-synovial: G1↑ ($p<0.05$)[4][8] G2↑ ($p<0.05$)[4][8]	
IL-10 Basal effects						
Alkatan et al., 2016 RCT	OAk (Sx + Rx) N= 48 (83.33%)[17] Female: 91.67% (NA) [1] Male: 8.33% (NA)[1] (G1) 61 ± 1 (G2) 59 ± 2	2/ (G1) Aerobic (i.e. cycling) (G2) Aerobic (i.e. swimming) From 20-30 min 3x/week at 40-50% HRR to 40-50min 3x/week at 60-70% HRR	12 wks	Plasma (T0) at baseline (T1) at week 12 (at least 48h after last exercise session)	**Within group differences: T0 vs T1** G1: ND: 152 ± (43) vs 155 ± (46) pg/mL[2] G2: ND: 105 ± (7) vs 95 ± (7) pg/mL[2]	ES_{G1}= 0.015 ES_{G2}= -0.319
Zhao et al., 2019 RCT	OAk (Sx) N= 55 Female: [4] Male: [4] (54-65)	2/ (G1): Strength (i.e. static low angle squat) (G2): Control (NA) 2x/day 30 min static low angle squats	2 y	Synovial fluid (OA knee) (T0) at baseline (T1) after 12 months	**Within group differences: T0 vs T1** G1: ↑: 45.14 ± 5.36 vs 90.45 ± 4.53 pg/mL ($p<0.001$)	ES_{G1}= 9.163
IL-10 Acute effects						
Helmark et al., 2010 RCT	OAk (Sx+Rx) N=31 (93.5%) Female: 100% (100%) (G1) 66 ±6 (G2) 67 ± 7	2/ (G1) Strength (G2) Control: non exercise 5 min warm-up on bicycle 25 sets of 10 repetitions at 60% of 1RM on legg-press machine	<24h (1x)	Synovial fluid: - peri-synovial - intra-articular At latest 1h after exercise; 6 samples every 30' (3h); samples were later pooled: T1= sample 1 to 3 (30', 60', 90') T2 = sample 4 to 6 (120', 150', 180')	**Between group differences G1 vs G2:** T1: ND[2][4] **Within group differences T1 vs T2:** Intra-articular: G1↑ ($p<0.05$)[4][8] G2: ND[2][4][8] peri-synovial: G1↑($p<0.05$)[4][8] G2: ND[2][4][8]	
IL-12 Basal effects						
Alkatan et al., 2016 RCT	OAk (Sx + Rx) N= 48 (83.33%)[17] Female: 91.67% (NA) [1] Male: 8.33% (NA)[1] (G1) 61 ± 1 (G2) 59 ± 2	2/ (G1) Aerobic (i.e. cycling) (G2) Aerobic (i.e. swimming) From 20-30 min 3x/week at 40-50% HRR to 40-50min 3x/week at 60-70% HRR	12 wks	Plasma (T0) at baseline (T1) at week 12 (at least 48h after last exercise session)	**Within group differences: T0 vs T1** G1: ND: 48 ± (8) vs 53 ± (10) pg/mL[2] G2: ND: 48 ± (10) vs 41 ± (7) pg/mL[2]	ES_{G1}= 0.124 ES_{G2}= -0.184
IL-13 Basal effects						
Alkatan et al., 2016 RCT	OAk (Sx + Rx) N= 48 (83.33%)[17] Female: 91.67% (NA) [1] Male: 8.33% (NA)[1] (G1) 61 ± 1 (G2) 59 ± 2	2/ (G1) Aerobic (i.e. cycling) (G2) Aerobic (i.e. swimming) From 20-30 min 3x/week at 40-50% HRR to 40-50min 3x/week at 60-70% HRR	12 wks	Plasma (T0) at baseline (T1) at week 12 (at least 48h after last exercise session)	**Within group differences: T0 vs T1** G1: ND: 50 ± (10) vs 46 ± (7) pg/mL[2] G2: ND: 37 ± (6) vs 31 ± (3) pg/mL[2]	ES_{G1}= -0.105 ES_{G2}= -0.298
TNF-α Basal effects						
Nicklas et al., 2004 RCT	OAk (Rx) N=316 (79.7%) Female[16]: 71.7% (NA)[1] Male[16]: 28.9% (NA)[1] (G1) 69 ± 6[6] (G2) 68 ± 7[6] (G3) 68 ± 5[6] (G4) 69 ± 6[6]	4/ (G1) Aerobic + strength (G2) Diet + aerobic + strength (G3) Diet (G4) Control: healthy lifestyle 1h, 3x/week: Aerobic (walking 15 min, 50-75%HRR), resistance training (2x12 repetitions, 15 min), aerobic (15 min), cooling down (15 min)	18 mo	Serum (T0) at baseline (T1) at 6 mo (T2) at 18 mo	**Between group differences: G1 vs G2 vs G3 vs G4** T0: ND: 3.4 ± 4.8 vs 3.4 ± 6.4 vs 2.5 ± 1.8 vs 3.8± 7.5 pg/mL[2] ΔTNF-α T0 - T1: -0.69 ± 5.8 vs -0.46 ± 3.7 vs -0.23 ± 1.8 vs -0.74 ± 3.7 pg/mL[2] T0 - T2: 0.28 ± 6.3 vs -0.72 ± 4.6 vs 0.64 ± 5.9 vs -0.77 ± 3.7 pg/mL[2] G1: ND (p=0.30) G3: ND (p=0.67) TNF-α/sTNFR1, TNF-α/sTNFR2, TNF-α/sTNFR1+sTNFR2 G1: ND[2] G2: ND[2]	
Samut et al., 2015 RCT	OAk (Sx+Rx) N=42 (95%) Female:90% (NA) Male: 10% (NA) (G1) 62.46± 7.71[6] (G2) 57.57 ± 5.79[6] (G3) 60.92 ± 8.85[6]	3/ (G1) Strength (isokinetic) (G2) Aerobic (G3) Control: info disease & recommendations precautions Strength: 3x/week	6 wks	Serum (T0) at baseline (T1) at 6 wks	**Between group differences: G1 vs G2 vs G3** T0: 0.446 (0.038 - 2.674) vs 1.310 (0.038 - 3.789) vs 0.038 (0.038 - 1.282) pg/mL, ($p<0.001$) T1: 0.223 (0.038 - 3.064) vs 0.753 (0.038 - 0.351) vs 0.038 (0.038 - 1.895) pg/mL[2]	

		5 min warm-up on treadmill 5 concentric flexion and extension exercises Aerobic: 3x/week 5 min warm-up on treadmill Week 1-4: 65-70% of age related HR Week 5-6: 70-75% of age related HR 5 min cool-down period			**Within goup differences: T0 vs T1** G1: ND (0.446 (0.038 - 2.674) vs 0.223 (0.038 - 3.064) pg/mL, (p=0.576) G2: ND (1.310 (0.038 - 3.789) vs 0.753 (0.038 - 0.351) pg/mL, (p=0.414) G3: ND (0.038 (0.038 - 1.282) vs 0.038 (0.038 - 1.895) pg/mL, (p=0.500)	
Kim et al., 2021 RCT	OAk (NA) N= 43 (98%) Female: 19 (44%) Male: 24 (56%) (G1) 67.4 ± 6.0 (G2) 66.9 ± 6.3	2/ (G1) Strength (i.e. aquatic exercises) (G2) Control: usual care + brochure on perioperative nutrition 3x/week 60 min until scheduled total knee arthroplasty surgery (4-8 weeks) 10 min warm-up, 20 min flexibility and strength, 20 min low intensity endurance, 10 min cool down. Resistance equipment (i.e. ankle cuffs) was added depending on the tolerance to increase intensity	4-6 wks	Blood (T0) at baseline (T1) 1 week before TKA surgery	**Between group differences: G1 vs G2** T0: ND: 1.38 ± 0.58 vs 2.85 ± 6.43 [2] **SMD:** Log(TNF-α): 0.10 [95% CI: -0.07 to 0.26] (p=0.25)	i.d.a.
Zhao et al., 2019 RCT	OAk (Sx) N= 55 Female: [4] Male: [4] (54-65)	2/ (G1): Strength (i.e. static low angle squat) (G2): Control (NA) 2x/day 30 min static low angle squats	2 y	Synovial fluid (OA knee) (T0) at baseline (T1) after 12 months	**Within group differences: T0 vs T1** G1: ↓: 22.43 ± 4.31 vs 14.07 ± 2.89 pg/mL (p<0.001)	ES_{G1}= -2.322
Gomes et al., 2012 NRUCT	OAk (Sx+Rx) N=15 (100%) Female: 100% (100%) 67 ± 4	1/ Aerobic 3x/week 5min warm up walking from 30 min (week 1) to 55 min (week 12) from 70% HRmax to 80% HRmax 5 min cool-down	12 wks	Plasma (T0) at baseline (T1) at 12 wks	**Within group differences:** T0 vs T1: ND; 37.8 ± 20.1[-6.0 to 81.63] vs 72.0 ± 105.2[8.4 to 135.6] pg/mL; (p>0.05); Cohen's d=0.638	ES_{G1}= 0.546
Alkatan et al., 2016 RCT	OAk (Sx + Rx) N= 48 (83.33%)[17] Female: 91.67% (NA)[1] Male: 8.33% (NA)[1] (G1) 61 ± 1 (G2) 59 ± 2	2/ (G1) Aerobic (i.e. cycling) (G2) Aerobic (i.e. swimming) From 20-30 min 3x/week at 40-50% HRR to 40-50min 3x/week at 60-70% HRR	12 wks	Plasma (T0) at baseline (T1) at week 12 (at least 48h after last exercise session)	**Within group differences: T0 vs T1** G1: ND: 264 ± (26) vs 253 ± (25) pg/mL[2] G2: ND: 246 ± (23) vs 236 ± (23) pg/mL[2]	ES_{G1}= -0.096 ES_{G2}= -0.097
Aguiar et al., 2014 NRUCT	OAk (Sx+Rx) N=27 (81.5%) Female: NA (81.8%) Male: NA (18.2%) 58.8 ± 6.4	1/ Other: Flexibility + strength 3x/week, 80min/day Supervised Week 1-4: 60% of max. load Week 5-8: 70% max. load Week 9-12: full weight determined by 10-MR test	12 wks	Serum (T0) at baseline (T1) at 12 wks Change after training	**Within group differences:** T0 vs T1: ND[2][4]; 113.05 [0 – 695.12] vs 127.57 [0 – 751.52] pg/mL[5][12]	ES_{T1-T0}= 0.080
TNF-α Acute effects						
Helmark et al., 2010 RCT	OAk (Sx+Rx) N=31 (93.5%) Female: 100% (100%) (G1) 66 ±6 (G2) 67 ± 7	2/ (G1) Strength (G2) Control: non exercise 5 min warm-up on bicycle 25 sets of 10 repetitions at 60% of 1RM on legg-press machine	<24h (1x)	Synovial fluid: - peri-synovial - intra-articular At latest 1h after exercise; 6 samples every 30' (3h); samples were later pooled: T1= sample 1 to 3 (30', 60', 90') T2 = sample 4 to 6 (120', 150', 180')	**Between group differences G1 vs G2:** T1: ND[2][4] **Within group differences T1 vs T2:** Intra-articular: G1↑ (p<0.05)[4][8] G2↑ (p<0.05)[4][8] peri-synovial: G1↑ (p<0.05)[4][8]	
Jayabalan et al., 2019 NRUCT	OAk (Sx+Rx) N= 27 (100%) Female: 74.07% (100%) Male: 25.93% (100%) 63.5 ± 7.7	2/ (G1) Aerobic: continuous walking (G2) Aerobic: interval walking 40-60% HRR; 1.3m/sec on treadmill	45 min (x2)	Serum (T0) at baseline (T1) after 15 min (T2) after 30 min (T3) after 45 min Sampling of interval walking was	**Between group differences G1 vs G2** T0: ND[8] 11.06 ± 7.10 vs 9.72 ± 6.38 ng/mL[2] T1: 9.66 ± 4.79 vs 9.31 ± 4.77 ng/mL[2] T2: 10.52 ± 5.55 vs 10.34 ± 5.21 ng/mL[2] T3: 10.93 ± 6.38 vs 10.24 ± 6.52 ng/mL[2]	

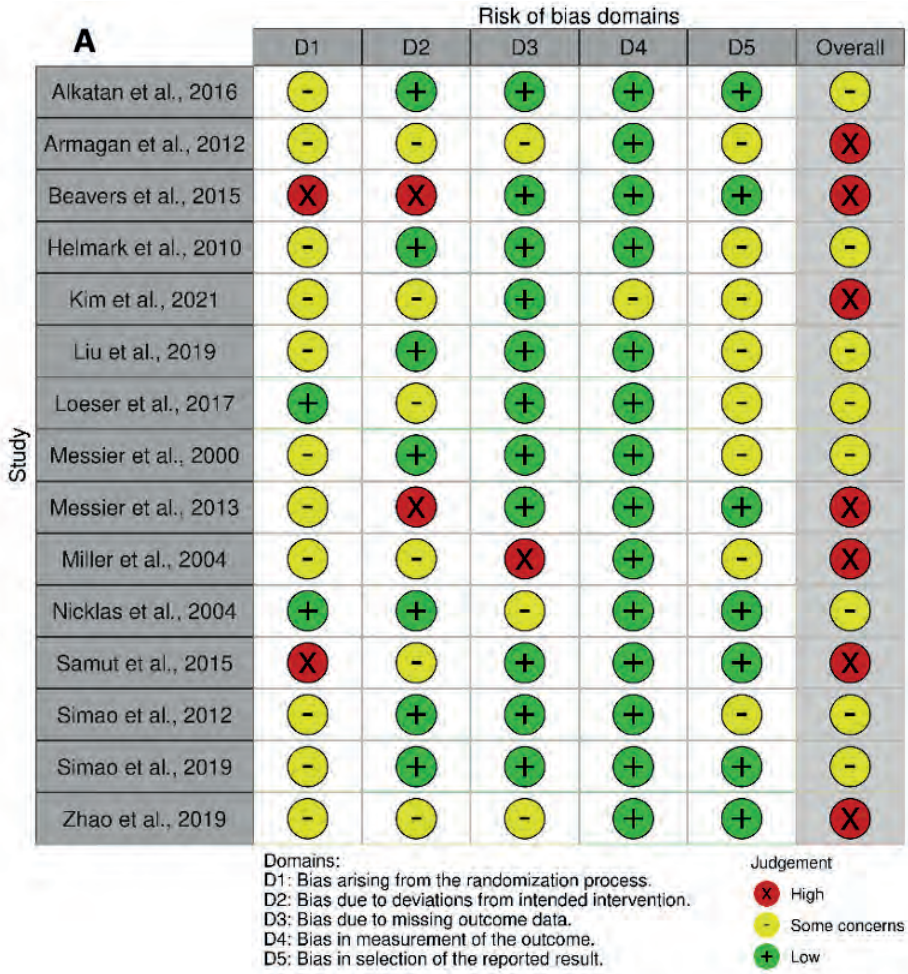
				performed at least 72h after sampling continuous walking	**Within group differences: T0 vs T1 vs T2 vs T3:** G1: 11.06 ± 7.10 vs 9.66 ± 4.79 vs 10.52 ± 5.55 vs 10.93 ± 6.38 ng/mL[2] G2: 9,72 ± 6.38 vs 9.31 ± 4.77 vs 10.34 ± 5.21 vs 10.24 ± 6.52 ng/mL[2] **Within group differences in increase from baseline** G1 : T1 vs T0: ND: (p=0.196) T2 vs T0: ND: (p=0.372) T3 vs T0: ND: (p=0.472) G2 : T1 vs T0: ND: (p= 0.387) T2 vs T0: ND: (p=0.339) T3 vs T0: ND: (p=0.385)	ES_{G1}= -0.019 ES_{G2}= 0.081
Gomes et al., 2012 NRUCT	OAk (Sx+Rx) N=15 (100%) Female: 100% (100%) 67 ± 4	1/ Aerobic 3x/week 5min warm up Walking from 30 min (week 1) to 55 min (week 12) from 70% HRmax to 80% HRmax 5 min cool-down	12 wks	Plasma (T0) at baseline: (T00) pre-acute EX (T01) post-acute EX (T02) 30' post-acute EX (T1) at 12 weeks: (T10) pre-acute EX (T11) post-acute EX (T12) 30' post-acute EX	**Within group differences:** ND: i.d.a. [2][4][11][18]	
sTNFR1 Basal effects						
Nicklas et al., 2004 RCT	OAk (Rx) N=316 (79.7%) Female[16]: 71.7% (NA)[1] Male[16]: 28.9% (NA)[1] (G1) 69 ± 6 (G2) 68 ± 7 (G3) 68 ± 5 (G4) 69 ± 6	4/ (G1) Aerobic + strength (G2) Diet + aerobic + strength (G3) Diet (G4) Control: healthy lifestyle 1h 3x/week: Aerobic (walking 15 min, 50-75%HRR), resistance training (2x12 repetitions, 15 min),	18 mo	Serum (T0) at baseline (T1) at 6 mo (T2) at 18 mo	**Between group differences: G1 vs G2 vs G3 vs G4** T0: ND: 1433 ± 404 vs 1395 ± 397 vs 1409 ± 470 vs 1464 ± 421 pg/mL[2] ΔsTNFR1 T0 - T1: -38 ± 224 vs -80 ± 226 vs -92 ± 290 vs -10 ± 291 pg/mL[2] T0 - T2: 25 ± 252 vs -3 ± 241 vs -34 ± 362 vs 62 ± 312 pg/mL[2] G1: ND, (p=0.54) G3↓ vs (G1 + G4) (Δlog sTNFR1:	
		aerobic (15 min), cooling down (15 min)			-0.070 ± (0.017) vs -0.013 ± (0.017) pg/mL), (p=0.007)	
Simao et al., 2012 RCT	OAk (Sx+Rx) N=35 (91.4%) Female: NA (87.5%) Male: NA (12.5%) (G1) 75 ± 7.4 (G2) 69 ± 3.7 (G3) 71 ± 5.3	3/ (G1) Strength (squat on vibration platform) (G2) Strength (squat) (G3) Control: no change of lifestyle 3x/week Volume of squat training was increased by increasing the time and number of sets and reducing rest time 10 min warm up on a bike at 70%HRmax	12 wks	Plasma (T0) at baseline (T1) at 12 wks	**Between group differences G1 vs G2 vs G3:** T0: 1033.0 «718-1281» vs 722.4 «522-1052» vs 682.8 «617-849» pg/mL[2] T1: 709.2 «379-1281» vs 742.5 «373 - 1492» vs 1072.0 «879 - 1252» pg/mL[2] **ΔPOST-hoc between:** G2 vs G1:ND: p>0.05 G3 vs G1↓: SD: p<0.01 G3 vs G2: ND: p>0.05	
					Within group differences Δ T0 vs T1 [13]: G1 ↓: -146.0 «-826 to 183»pg/mL. (p=0.04) G2: ND: 155.0 «-720 to 536» pg/mL, (p=0.95) G3 ↑: 389.2 «102 to 638» pg/mL, (p=0.001)	
Gomes et al., 2012 NRUCT	OAk (Sx+Rx) N=15 (100%) Female: 100% (100%) 67 ± 4	1/ Aerobic 3x/week 5min warm up Walking from 30 min (week 1) to 55 min (week 12) from 70% HRmax to 80% HRmax 5 min cool-down	12 wks	Plasma (T0) at baseline (T1) at 12 wks	**Within group differences:** T0 vs T1↑; 540.7 ± 289.1[380.6 to 700.8] vs 1120.0 ± 209.2 [999.2 to 1241.0] pg/mL (p<0.001); Cohen's d=3246	ES_{G1}= 2.325
Aguiar et al., 2014 NRUCT	OAk (Sx+Rx) N=27 (81.5%) Female: NA (81.8%) Male: NA (18.2%) 58.8 ± 6.4	1/ Other: Flexibility + strength 3x/week, 80min/day Supervised Week 1-4: 60% of max. load Week 5-8: 70% max. load Week 9-12: full weight determined by 10-MR test	12 wks	Serum (T0) at baseline (T1) at 12 wks (change after training)	**Within group differences:** T0 vs T1: ND[2][4]; 1275.32 [119.53-2329.49] vs 1257.97 [7.46 – 2002.58] pg/mL[5][16]	ES_{T1-T0}= -0.033
sTNFR1 Acute effects						
Gomes et al., 2012 NRUCT	OAk (Sx+Rx) N=15 (100%) Female: 100% (100%) 67 ± 4	1/ Aerobic 3x/week 5min warm up Walking	12 wks	Plasma (T0) at baseline: (T00) pre-acute EX (T01) post-acute	**Within group differences:** T0: T00 vs T01 vs T02[4] 538.46 [163.46-1028.85] vs 1278.85 [413.46-1971.15] vs 1115.38 [461.54-1961.54] pg/mL[5] T00 vs T01↑ (p<0.001)	

		from 30 min (week 1) to 55 min (week 12) from 70% HRmax to 80% HRmax 5 min cool-down		EX (T02) 30' post-acute EX (T1) at 12 weeks: (T10) pre-acute EX (T11) post-acute EX (T12) 30' post-acute EX	T00 vs T02↑ (p<0.001) T01 vs T02: ND[2] T1: T10 vs T11 vs T12[4] 1125 [788.46-1519.23] vs 951.92 [375-1375] vs 721.15 [153.85-1307.69] pg/mL[5] T10 vs T12↓ (p<0.01) T11 vs T12↓ (p<0.05) T0 vs T1: T01 vs T11↓ (p<0.05) T02 vs T12↓ (p<0.01)	
sTNFR2 Basal effects						
Nicklas et al., 2004 RCT	OAk (Rx) N=316 (79.7%) Female[16]: 71.7% (NA)[1] Male[16]: 28.9% (NA)[1] (G1) 69 ± 6[6] (G2) 68 ± 7[6] (G3) 68 ± 5[6] (G4) 69 ± 6[6]	4/ (G1) Aerobic + strength (G2) Diet + aerobic + strength (G3) Diet (G4) Control: healthy lifestyle 1h, 3x/week: Aerobic (walking 15 min, 50-75%HRR), resistance training (2x12 repetitions, 15 min), aerobic (15 min), cooling down (15 min)	18 mo	Serum (T0) at baseline (T1) at 6 mo (T2) at 18 mo	**Between group differences: G1 vs G2 vs G3 vs G4** T0: ND: 2760 ± 807 vs 2656 ± 792 vs 2674 ± 842 vs 2850 ± 1127 pg/mL[2] ΔsTNFR2 T0 - T1: -148 ± 409 vs -78 ± 427 vs -53 ± 547 vs -59 ± 915 pg/mL[2] T0 - T2: 24 ± 514 vs -61 ± 460 vs 38 ± 665 vs 38 ± 561 pg/mL[2] G1: ND (p=0.08) G3: ND (p=0.23)	
Simao et al., 2012 RCT	OAk (Sx+Rx) N=35 (91.4%) Female: NA (87.5%) Male: NA (12.5%) (G1) 75 ± 7.4 (G2) 69 ± 3.7 (G3) 71 ± 5.3	3/ (G1) Strength (squat on vibration platform) (G2) Strength (squat) (G3) Control: no change of lifestyle 3x/week Volume of squat training was increased by increasing the time and number of sets and reducing rest time 10 min warm up on a bike at 70%HRmax	12 wks	Plasma (T0) at baseline (T1) at 12 wks	**Between group differences G1 vs G2 vs G3:** T0: 4944.0 «3819-5862» vs 4141.0 «3329 - 4993» vs 3568.0 «3319 - 5002» pg/mL; (p=0.20) T1: 3987.0 «2964 - 5067» vs 3650.0 «2949 - 4719» vs 4673.0 «3266 - 5371» pg/mL **ΔPOST-hoc between:** G2 vs G1: ND: p>0.05 G3 vs G1↓: p<0.01 G3 vs G2: ND: p>0.05	
					Within group differences ΔT0 vs T1 [13]: G1↓: -996.5 «-1750 to 147.3» pg/mL, (p=0.005) G2: ND: -497.7«-1351 to 2219» pg/mL, (p=0.38) G3: ND: 35.4 «-1206 to 2104» pg/mL (p=0.58)	
Gomes et al., 2012 NRUCT	OAk (Sx+Rx) N=15 (100%) Female: 100% (100%) 67 ± 4	1/ Aerobic 3x/week 5min warm up Walking from 30 min (week 1) to 55 min (week 12) from 70% HRmax to 80% HRmax 5 min cool-down	12 wks	Plasma (T0) at baseline (T1) at 12 wks	**Within group differences:** T0 vs T1↓; 4542.8 ± 1688 [3608.0 to 5478.0] vs 3177.5 ± 1050.0 [2596.0 to 3759.0] pg/mL, p<0.001); Cohen's d=1373	ES_{G1}= -0.997
Aguiar et al., 2014 NRUCT	OAk (Sx+Rx) N=27 (81.5%) Female: NA (81.8%) Male: NA (18.2%) 58.8 ± 6.4	1/ Other: Flexibility + strength 3x/week, 80min/day Supervised Week 1-4: 60% of max. load Week 5-8: 70% max. load Week 9-12: full weight determined by 10-MR test	12 wks	Serum (T0) at baseline (T1) at 12 wks (change after training)	**Within group differences:** T0 vs T1: ND[2][4]; 4431.81 [1909.77-6489.78] vs 4436.81 [1640.90 – 6589.61] pg/mL[5][12]	ES_{T1-T0}= 0.004
sTNFR2 Acute effects						
Gomes et al., 2012 NRUCT	OAk (Sx+Rx) N=15 (100%) Female: 100% (100%) 67 ± 4	1/ Aerobic 3x/week 5min warm up Walking from 30 min (week 1) to 55 min (week 12) from 70% HRmax to 80% HRmax 5 min cool-down	12 wks	Plasma (T0) at baseline: (T00) pre-acute EX (T01) post-acute EX (T02) 30' post-acute EX (T1) at 12 wks: (T10) pre-acute EX (T11) post-acute EX (T12) 30' post-acute EX	**Within group differences:** T0: T00 vs T01 vs T02[4] 4562.25 [2795.18-7967.87] vs 3020.08 [1574.30-4722.89] vs 2923.69 [1542.17-5076.31] pg/mL[5] T00 vs T01↓ (p<0.001) T00 vs T02↓ (p<0.001) T01 vs T02: ND[2] T1: T10 vs T11 vs T12[4] 3180.72 [1863.45-5172.69] vs 2795.18 [1156.63-4755.02] vs 2602.41 [1124.50- 4176.71] pg/mL[5] T10 vs T12↓ (p<0.05) T11 vs T12: ND[2]	
Leptin Basal effects						
Miller et al., 2004 RCT	OAk (Sx) N= 309 (97.78%) Female[16]: 73.11% (NA) Male[16]: 26.89% (NA) (G1) 69.1 ± 6.5 (G2) 68.7 ± 6.2 (G3) 68.7 ±6.7 (G4) 67.8 ±6.5	4/ (G1) Exercise (aerobic + strength) (G2) Healthy lifestyle (G3) Diet + exercise (aerobic + strength) (G4) Dietary weight loss	18mo	Serum (T0) at baseline (T1) at 6 mo (T2) at 18 mo	**Within group: T0 vs T1 vs T2** 31.7 ± 19.0 vs 32.0 ± 21.5 vs 29.9 ± 23.0 ng/mL[2] No main exercise effect	

		Exercise content: 60 min, 3x/week 5 min warm-up, 15 min aerobic exercises (50-85% HRR), 20 min strength exercises, 15 min aerobic exercises (50-85% HRR), 5 min cool-down phase				
BDNF Basal effects						
Simao et al., 2019 RCT	OAk (Sx+Rx) N= 15 Female: 100% (100%) (G1) 75 (68.5-81.5) yrs (G2) 71 (67.7-74.3) yrs	2/ (G1) Strength+ whole body vibration + squat exercises (G2) Strength 3x/week 10 min warm-up on cycle at 70% predicted HRmax Intensity of squats was increased by increasing the number of repetitions (6x20s to 8x40s) and reducing resting time (40-25s)	12 wks	Plasma (T0) prior to the intervention 24h after intervention period (T1) at 12 wks	**Between group differences: G1 vs G2** T0: ND: 4778 [2952.4-6603.7] vs 3043 [1623.4-4462.6] pg/mL, (p=0.06) **Delta analysis (pretest-posttest)** G1: 122.1 [13] ± (741.94) [5] pg/mL [2] G2: -2037 [13] ± (612.9) [5] pg/mL [2] G1 ↑ vs G2 ($p \leq 0.05$; effect size: 1.1)	
Gomes et al., 2014 NRUCT	OAk (Sx+Rx) N=16 (100%) Female: 100% (100%) 67 ± 4.41 yrs	1/ Aerobic 3x/week walk training (aquatic and land), 30 min at 70% HR max	12 wks	Plasma (T0) at baseline (T1) at 12 weeks	**Within group differences :** T0 vs T1↑; 8343± 3690 vs 14027 ± 4361 pg/mL, (p<0.001)	ES= 1.412
Liu et al., 2019 RCT	OAk (Sx + Rx) N= 140 (77.14%) Female: 76.85% (NA) Male: 23.15% (NA) (G1) 40-70 yrs (G2) 40-68 yrs (G3) 40-70 yrs (G4) 40-70 yrs	4/ (G1) Other: Tai Chi (G2) Other: Baduanjin (G3) Aerobic: Cycling (70-75% max HR) (G4) Control: basic health education 1x/week (G1)+(G2) 5x/week 60 min: 10 min warm-up, 30 min exercise (70-75% max HR), 10 min breathing techniques, 10 min relaxation Under supervision	12 wks	Serum (T0) at baseline (1week before intervention) (T1) after 12wks (within 1 week after finishing intervention)	**Between group differences:** G1 vs G2 vs G3 vs G4 T0: ND: 50.8 (3.8 -173.0) vs 45.4 12.5 – 134.5) vs 31.3 (1.6 – 497.2) vs 54.9 (22.4 – 389.9), (p=0.32) T1-T0: ND: 3.0 ± 42.3 vs 14.6 ± 60.2 vs 1.1 ± 101.5 vs -14.0 ± 50.0, (p= 0.46) **ΔPost-hoc between** G1 vs G4 T1-T0: ND: p= 0.90 G2 vs G4 T1-T0: ND: p= 0.51 G3 vs G4 T1-T0: ND: p= 0.92 G1 vs G2 T1-T0: ND: p= 0.99 G1 vs G3 T1-T0: ND: p= 1.00 G2 vs G3 T1-T0: ND: p= 0.99	ES_{G1-G4}= -0.370 ES_{G2-G4}= -0.512 ES_{G1-G4}= -0.185
BDNF Acute effects						
Gomes et al., 2014 NRUCT	OAk (Sx+Rx) N=16 (100%) Female: 100% (100%) 67 ± 4.41 yrs	1/ Aerobic 2 min warming up walking on treadmill at 1mph 18 min walking on treadmill at 2mph	12 wks	Plasma (T0) at baseline: (T00) pre-acute EX (T01) post-acute EX (T02) 30' post-acute EX (T1) at 12 wks: (T10) pre-acute EX (T11) post-acute EX (T12) 30' post-acute EX	**Within group differences:** T0: T00 vs T01 vs T02 7.693 ± 4.454 vs 12.242 ± 3.806 vs 11.190 ± 3.847 pg/mL T00 vs T01↑ (p<.0001) T00 vs T02↑ (p<0.001) T1: ND [2][4][18]	$ES_{T01-T00}$= 1.101 $ES_{T02-T00}$= 0.843

BDNF: Brain Derived Neurotrophic Factor; **CRP**= C-reactive Protein; **EX**= exercise; **(G)**= group; **IL**= Interleukin; **IL-6sR**=soluble interleukin 6 receptor; **info & rec**= information and recommandations; **mo**=mon Difference; **OAk**=Osteoarthritis knee; **Rx**=radiographic; **sTNFR1**= soluble Tumor Necrosis Factor-α Receptor 1; **sTNFR2**= soluble Tumor Necrosis Factor-α Receptor 2; **Sx**=symptomatic; **TGF-1β**= Transformin **α**=Tumor Necrosis Factor-α; **wks**=weeks; ; **WL**= weight loss; **LE OA**: lower extremities (knee, hip, lower back, ankle) osteoarthritis; **OAh**: hip osteoarthritis; [1]Based on available samples at baseline; [2] p valu visit, gender, baseline BMI, baseline biomarker value; [4] no data reported; [5] results based on graph measurement using GetData Graph Digitizer; [6] range: group with lowest mean age vs group with high sensitivity C-Reactive Protein; [8] interpretation of graphs and text; [9] adjusted for baseline BMI, sex, baseline values(significance level set at .025); [10] significance level set at .008; [11] no graphs; [12]only levels below minimum detectable concentration; [13] p values/concentrations obtained from author; [14] mean; [15] adjusted for baseline values, age, gender and BMI; [16]obese; [17]intention to treat analysis [19]median; [20] adjusted for baseline values baseline BMI and gender; [21] adjusted for age and baseline BMI; ↑ = significant increase; ↓= significant decrease; SD: significant difference; i.d.a.: insufficient dat (before–after); **1x=** one session; mean± SD; mean± (SE); median (minimum - maximum); median «interquartil range»; mean[minimum - maximum]; mean ± SD[95% CI: ...to...]; mean[95% CI: ..,to..,]

Table 4:
Risk of Bias. 4A) Risk of bias in RCTs. 4B) Risk of bias in NR(U)CTs



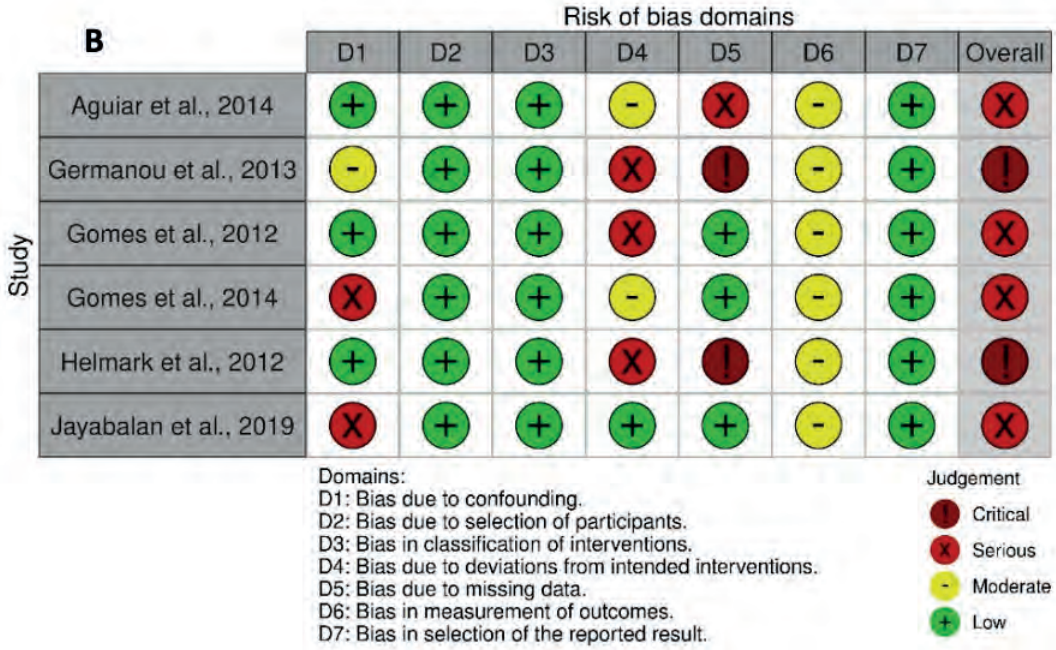


Table 5:
Schematic summary of the findings per biomarker

Biomarkers with a pro-inflammatory character:			
Biomarker	**BE/AE**	**Type analysis**	**Effect exercise therapy**
CRP	BE	*Meta-analysis*	↓ (Samut et al.,2015; Nicklas et al.,2004)
		Training vs. control group	↔ (Kim et al.,2021)
		Within training group	↔ (Germanou et al., 2013), ↔ (Beavers et al., 2015)
	AE	*Within training group*	↔ (Germanou et al.,2013)
CRPM	BE	*Within training group*	↔ (Loeser et al., 2017)
IL-1β	BE	*Within training group*	↔ (Alkatan et al., 2016; Armagan et al.,2012) ↓° (Zhao et al., 2019; Messier et al., 2000)
IL-5	BE	*Within training group*	↔ (Alkatan et al., 2016)
IL-6	BE	*Meta-analysis*	↔ (Samut et al., 2015; Nicklas et al.,2004)
		Training vs. control group	↔ (Kim et al., 2021)
		Within training group	↔ (Messier et al;,2013), ↔ (Germanou et al., 2013 ; Gomes et al.,2012), ↓ (Alkatan et al.,2016), ↓ (Aguiar et al.,2015)
IL-6sR	BE	*Within training group*	↔ (Nicklas et al.,2004)
IL-7	BE	*Within training group*	↔ (Alkatan et al., 2016)
IL-8	BE	*Within training group*	↔ (Alkatan et al.,2016)
	AE	*Training vs. control group*	↔° (Helmark et al.,2010)
		Within training group	↑°(Helmark et al.,2010)
IL-12	BE	*Within training group*	↔ (Alkatan et al.,2016)

TNF-α	BE	*Meta-analysis*	↓ (Samut et al.,2015; Nicklas et al.,2004)
		Training vs. control group	↔ (Kim et al.,2021)
		Within training group	↓° (Zhao et al., 2019), ↔ (Gomes et al., 2012 ; Aguiar et al.,2015), ↔ (Alkatan et al., 2016)
	AE	*Training vs. control group*	↔° (Helmark et al., 2010)
		Within training group	↑° (Helmark et al., 2010), ↔ (Jayabalan et al., 2019), ↔ (Gomes et al.,2012)
Leptin	BE	*Within training group*	↔ (Miller et al., 2004)

Biomarkers with an anti-inflammatory character:			
Biomarker	**BE/AE**	**Type analysis**	**Effect exercise therapy**
IL-2	**BE**	*Within training group*	↔ (Alkatan et al., 2016)
IL-4	**BE**	*Within training group*	↔ (Alkatan et al., 2016)
IL-6	**AE**	*Training vs. control group*	↔°(Helmark et al., 2010)
		Within training group	↑(Germanou et al., 2013), ↑°(Helmark et al., 2010), ↔° (Helmark et al., 2012), ↔ (Gomes et al., 2012)
IL-10	**BE**	*Within training group*	↔ (Alkatan et al., 2016), ↑° (Zhao et al., 2019)
	AE	*Training vs. control group*	↔° (Helmark et al., 2010)
		Within training group	↑° (Helmark et al., 2010)
IL-13	**BE**	*Within training group*	↔ (Alkatan et al., 2016)
sTNFR1	**BE**	*Meta-analysis*	↔ (Nicklas et al., 2004; Simao et al., 2012)
		Within training group	↔ (Simao et al., 2012), ↑(Gomes et al., 2012), ↔(Aguiar et al., 2015)
	AE	*Within training group*	↑(Gomes et al., 2012), ↓(Gomes et al., 2012)

sTNFR2	**BE**	*Meta-analysis*	**↔ (Nicklas et al., 2004; Simao et al., 2012)**
		Within training group	**↔(Simao et al.,2012)**, ↓(Gomes et al.,2012), ↔(Aguiar et al.,2015)
	AE	*Within training group*	↓(Gomes et al.,2012)
BDNF			
Biomarker	**BE/AE**	**Type analysis**	**Effect exercise therapy**
BDNF	**BE**	*Training vs. control group*	**↔(Liu et al., 2019)**
		Within training group	↑(Gomes et al., 2014)
	AE	*Within training group*	↑(Gomes et al., 2014), ↔ (Gomes et al., 2014)

'↑' indicates an increase , '↓' indicates a decrease, '↔' indicates no change, arrow written in bold indicates evidence based on RCT, arrow in grey indicates evidence based on NR(U)CT

BE : basal effect ; AE : acute effect A: aerobics; S: strength training; IL: interleukin, sTNFR: soluble tumor necrosis factor receptor, BDNF: brain derived neurotrophic factor, TNF: tumor necrosis factor, CRP: C-reactive protein

A Scoping Review on the Effects of Physical Exercise and Fitness on Peripheral Leukocyte Energy Metabolism in Humans

Charles F Hodgman[1], Rebekah M Hunt[1], Justin C Crane[1], Mahmoud T Elzayat[1], Emily C LaVoy1[*]

[1]Univeristy of Houston Department of Health and Human Performance

ABSTRACT

Background: Both acute and chronic exercise have profound effects on systemic metabolism and the immune system. While acute exercise transiently disturbs energy homeostasis and elicits acute inflammation, exercise training improves systemic metabolic capacity, lowers basal inflammation, and reduces infection risk. Accordingly, accumulating evidence indicates links between systemic and immune cell metabolism and suggests that cellular metabolism may be an important way exercise influences immune function. Yet, no reviews have systematically surveyed the literature in this area. Aims: The aims of this scoping review were to collect, summarize, and provide descriptive analysis of literature on the effects of acute exercise, chronic exercise, and physical fitness on peripheral leukocyte energy metabolism of human adults. Methods: Reports were retrieved from the databases Pubmed, Scopus, and Embase and hierarchically filtered for eligibility. Eligible reports were those that implemented acute or chronic exercise interventions, or assessed physical fitness, in relation to the regulation or function of leukocyte energy metabolism in human adults. Data were charted from eligible reports by two independent reviewers, confirmed by conference, and organized for reporting. Results & Conclusion: Results suggest acute exercise can influence the regulation and function of leukocyte metabolism, with some similarities to what has been previously documented in skeletal muscle. Data also evidence that exercise training and/or physical fitness alters cellular metabolic regulation and function. Improvements in markers of cell respiratory function or mitochondrial regulation were frequently observed following training or with greater fitness. However, notable gaps in the literature remain. These gaps include: the effects of acute exercise and exercise training on leukocyte glycolysis, the effects of resistance and concurrent exercise, and potential differences in the effects of exercise between immune cell types and subsets. Future research is encouraged to fill the latter gaps and further delineate how exercise influences the immune system and can be used to support overall health.

Keywords: Exercise, Physical Fitness, Human, Leukocyte, Metabolism

**Correspondence to:*
Emily C. LaVoy
Associate Professor
Department of Health and Human Performance
3875 Holman Street Room 104 Garrison Hall
Houston, TX USA 77204
(713) 743-0829, eclavoy@uh.edu

INTRODUCTION

Physical exercise is essential to maintaining or improving physical and physiological function and preventing disease. Exercise training is a preventative measure against the development of multiple chronic diseases (8), and higher levels of both cardiorespiratory fitness and physical strength are negatively correlated with all-cause mortality (37, 39). These beneficial effects appear to arise from compensatory responses elicited by the stress of each exercise bout, that summate to enhanced function (30). While acute exercise results in metabolic perturbation proportional to its intensity and duration, training leads to improved efficiency of and capacity for energy production that lessens the stress of a given workload. This has been well demonstrated both systemically and at the level of skeletal muscle (30, 32). Similarly, acute exercise provokes an inflammatory immune response that scales with workload (i.e., greater inflammation following prolonged and intense exercise), while exercise training diminishes basal inflammation and risk for infection (71).

The parallel effects of exercise on energy metabolism and the immune system are particularly intriguing given the profound role of cellular metabolism in determining immune cell phenotype and function (52). Broadly, quiescent, regulatory, and memory cells are fueled by mitochondrial oxidation of fatty acids and carbohydrates, while activated and effector cells are biased towards the use of glycolysis (52). Stimulation of macrophages (e.g., with lipopolysaccharide) and T cells (e.g., with anti-CD3 and anti-CD28 antibodies) shifts metabolic poise from mitochondrial respiration to glycolysis, ostensibly to fuel inflammatory processes and/or proliferation (45, 52, 60). In contrast, mitochondrial fatty acid oxidation is characteristic of anti-inflammatory (i.e., M2-like) macrophages and regulatory T cells, and appears to constrain inflammation when enforced (45, 68). However, mitochondrial respiration is not the strict domain of quiescent and regulatory cells. Mitochondrial respiration also appears necessary for initial T cell activation, memory transition, and persistent function (9, 25, 62, 72). Likewise, inflammatory (i.e., M1-like) macrophages utilize mitochondrial flux to generate inflammatory cytokines and reactive oxygen species (ROS) (46). Together, data collected to date emphasize fundamental links between cellular metabolic programs and immune cell form and function.

A variety of methods exist for investigating cellular metabolism, including within cells of the immune system. Though complete survey of these methods is beyond the scope of this review, a brief summary will be provided to aid interpretation of the current results. Those interested in additional detail may find references (5), (35), (54), (55), and (70) useful. In general,

measures of cellular metabolism may be categorized as either assessing metabolic regulation or metabolic function. Metabolic regulation includes assessments of gene and protein-level expression for components of fuel oxidation and ATP generation, as well as assessments of the state of cell mitochondria. Examples include gene or protein-level expression of targets regulating glycolysis (e.g., hexokinase), cellular respiration (e.g., cytochrome c oxidase), and mitochondrial homeostasis (e.g., mitofusin). Assessments of mitochondrial mass and membrane polarity contribute information on cells' metabolic state and capacity for mitochondrial respiration. Measures of metabolic function include assays of enzyme activity (e.g., lactate dehydrogenase), fuel oxidation and generation of intermediates (e.g., fatty acid oxidation), as well as real-time assessments of metabolic pathway function (e.g., cellular respiration). Common platforms for probing cellular metabolic function include Agilent Seahorse Analyzers and Oroboros' O2k-Respirometer. Both estimate cellular metabolic function, over time and in response to various metabolic inhibitors, through measurement of oxygen consumption and/or proton efflux to provide evidence of mitochondrial respiration and glycolytic flux.

The acute and chronic effects of exercise on systemic and cellular metabolism suggest that cellular metabolic changes may be an important means by which exercise alters immune function. For instance, the coincident mobilization of energy substrates (e.g., glucose from liver, fatty acids from adipose tissue) and leukocytes during acute exercise may both bias the mobilized leukocytes towards inflammatory action and support effector cell function fueled by glycolysis (28, 52, 61). In contrast, oxidative adaptations similar to those observed in skeletal muscle could support memory cell formation following pathogen encounter, as well as promote anti-inflammatory cell function (43, 46, 72). Recent publications support the hypothesis that differences in immune function associated with exercise and physical fitness are related to cellular metabolic changes (6, 41). Yet, to our knowledge no reviews have yet surveyed the literature regarding the metabolic effects of exercise within the immune system. Consequently, the aim of this scoping review is to collate, summarize, and provide a descriptive analysis of research on the effects of acute exercise and chronic exercise on leukocyte energy metabolism. Relatedly, we also sought to summarize research on the relationships between physical fitness and leukocyte energy metabolism.

METHODS

Overview

This review adopted the scoping review framework proposed by Arksey and O'Malley (4). Namely, we: 1) identified the research question, 2) identified relevant literature, 3) reviewed and selected studies, 4) charted the data, 5) collated and summarized the results for reporting. The review was guided by the research question, "What is known about the effects of acute exercise, chronic exercise, and physical fitness on peripheral blood leukocyte metabolism in human adults?" For the purpose of the review, the effects of acute exercise were defined as those observed within 24 hours of an exercise bout, while the effects of chronic exercise were those observed at rest following >10 days of exercise training. Measures of physical fitness included the application of any standardized test of participant strength and/or cardiorespiratory fitness. Leukocyte energy metabolism included regulatory and/or functional assessments of cellular glycolysis and mitochondrial respiration within peripheral leukocytes. Given the relationship between leukocyte metabolism and function, measures of leukocyte function were also included. Measures of cell function included the assessment of cell activation, motility, proliferation, and cytokine or ROS production.

Literature Search Strategy

To confirm the necessity of the project, the databases Pubmed, Cochrane Library, and JBI Evidence Synthesis were searched to identify overlapping reviews (56). This search returned 0 reviews through JBI Evidence Synthesis, 0 reviews through Cochrane Library, and 56 results in Pubmed. Nineteen of the latter results were deemed potentially relevant to our research question. However, following manual review, none of the nineteen results possessed overlapping objectives to our own and this was taken

Table 1: Search Strategy Employed in Each Database

Database	Search Terms
PubMed	("cardiorespiratory fitness"[MESH] OR "cardiorespiratory fitness"[tiab] OR "Physical Fitness"[Mesh] OR "physical fitness"[tiab] OR "Physical Endurance"[Mesh] OR "physical endurance"[tiab] OR "High-Intensity Interval Training"[Mesh] OR "High-Intensity Interval Training"[tiab] OR HIIT[tiab] OR "Exercise"[Mesh] OR exercise[tiab] OR "acute exercise"[tiab]) AND ("Leukocytes"[Mesh] OR Leukocytes[tiab] OR "Leukocytes, Mononuclear"[Mesh] OR "Mononuclear Leukocytes"[tiab] OR "peripheral blood mononuclear cells"[tiab] OR "Lymphocytes"[Mesh] OR lymphocytes[tiab] OR PBMC*[tiab]) AND ("Oxygen Consumption"[Mesh] OR "Oxygen Consumption"[tiab] OR "Cell Respiration"[Mesh] OR "Cellular Respiration"[tiab] OR "Energy Metabolism"[Mesh] OR "Energy Metabolism"[tiab] OR "Oxidative Phosphorylation"[Mesh] OR "Oxidative Phosphorylation"[tiab] OR "Glycolysis"[Mesh] OR Glycolysis[tiab] OR "Mitochondrial Dynamics"[Mesh] OR "Mitochondrial metabolism"[tiab] or bioenergetic*[tiab])
Scopus	(TITLE-ABS-KEY (*"cardiorespiratory fitness"* OR *"physical fitness"* OR *"physical endurance"* OR *"High-Intensity Interval Training"* OR *exercise* OR *"acute exercise"*) AND TITLE-ABS-KEY (*leukocyte** OR *"peripheral blood mononuclear cells"* OR *lymphocyte** OR *pbmc**) AND TITLE-ABS-KEY (*"Oxygen Consumption"* OR *"Cell Respiration"* OR *"Cellular Respiration"* OR *"Energy Metabolism"* OR *"Oxidative Phosphorylation"* OR *glycolysis* OR *"mitochondrial dynamics"* OR *bioenergetic**))
Embase	(exp cardiorespiratory fitness/ or exp fitness/ or exp endurance/ or exp high intensity interval training/ or exp exercise/) and (exp leukocyte/ or exp mononuclear cell/ or exp peripheral blood mononuclear cell/ or exp lymphocyte/) and (exp oxygen consumption/ or exp cell respiration/ or exp energy metabolism/ or exp oxidative phosphorylation/ or exp glycolysis/ or exp mitochondrial dynamics/ or exp mitochondrial respiration/)

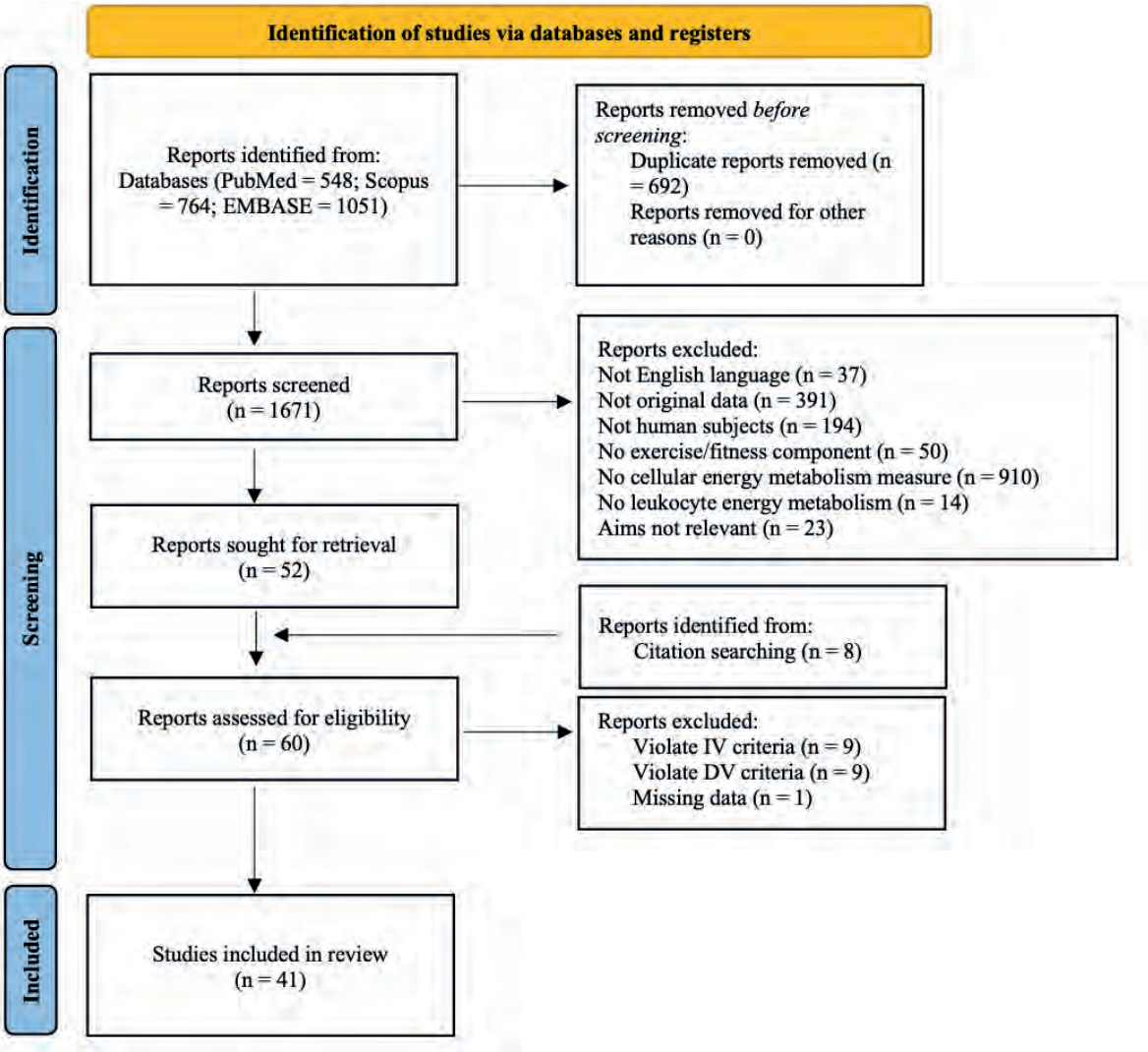


Figure 1:
Modified From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71
For more information, visit: http://www.prisma-statement.org/

as justification for the current review (Supplementary Table 1).

Relevant literature was sought through the online databases PubMed, Scopus, and Embase, and review of references within selected reports. The search strategy for each database was developed between the first and last author and refined through consultation with the department librarian. The searches were designed to retrieve reports on the effects of exercise or fitness on the regulation and function of energy metabolism of peripheral leukocytes in human adults. Reports published prior to May 9, 2022 (the date of search) were considered. The search strategy for each database is listed in Table 1.

Reference Selection

Following database search, all results were compiled within a digital reference manager (Mendeley) and hierarchically filtered for relevance by two independent reviewers (CFH and MTE). Reports were evaluated for inclusion through review of bibliographic data, abstracts, and full-text as necessary. Reports were excluded if they: were duplicates, were not published in English, did not report original data (e.g., review, opinion), did not include human participants, did not quantify or classify exercise/fitness/physical activity, or did not include measures of leukocyte metabolic regulation or function. Additionally, reports were screened according to their objectives and whether outcome measures were used to assess metabolic function as opposed to other aims (e.g., mitochondrial membrane depolarization as indication of cell viability versus respiratory function). Reports deemed potentially relevant were independently reviewed in full by two reviewers (CFH and RMH), followed by conference to confirm relevance. Full-texts of potentially relevant reports identified through citation searching were also retrieved for review. Reports no longer deemed relevant following review and conference were excluded (Supplementary Table 2). No conflicts in estimation of study relevance arose between reviewers.

Data Charting

An original data charting tool was developed based on data expected to be reported, and desired for inclusion in the review. A draft of this tool was piloted against three recent reports that investigated effects of acute exercise, effects of acute and chronic exercise, or effects of physical fitness on leukocyte metabolism by the reviewers listed above. Charted data were compared between reviewers following the pilot, and the tool was then

revised to economize and standardize the charting process. A blank version of the final charting tool is available upon request. Following completion of data charting for all reports, data from each reviewer were assembled for comparison. Conflicts in the charted data were reconciled through review of the associated report. Remaining disagreement regarding interpretation of the data were resolved through discussion. The final data selected for reporting represent consensus of both reviewers.

Data Collation and Reporting

Within each report, charted data were summarized to relate key study characteristics, findings, and conclusions. Across reports, data were categorized and aggregated hierarchically according to: effect(s) investigated (acute exercise, chronic exercise, physical fitness), intensity/type of exercise or training (heavy-severe intensity aerobic, light-moderate intensity aerobic, concurrent exercise, resistance exercise), participant population(s) (athletes, healthy active, inactive and/or presence of disease), cell population (mixed cell populations, isolated cell populations), and outcomes (metabolic regulation, metabolic function, cell function).

Investigated effect(s) were categorized based on whether a single bout and/or a period of training were implemented, or cross-sectional comparisons of fitness were performed. The intensity or type of exercise was classified using the described methods into heavy-severe intensity aerobic, light-moderate intensity aerobic, concurrent exercise, or resistance exercise categories. Heavy-severe intensity aerobic exercise includes those reports which implemented acute aerobic exercise or a training program, the majority of which was in or above the heavy intensity domain. The heavy intensity domain was defined as greater than the first ventilatory threshold (i.e., >75-80% maximum heart rate, >60-65% VO_{2max}) (42, 57, 58, 63). Light-moderate intensity aerobic exercise includes those reports which implemented acute exercise or a training program, the majority of which was within or below the upper limit of the moderate intensity domain (i.e., below the lower limit of heavy intensity) (11, 57). Thus, reports classified as implementing light-moderate intensity aerobic exercise were those that occurred below the thresholds listed for heavy-severe intensity (e.g., <75-80% maximum heart rate). Concurrent exercise includes reports that implemented exercise or a training program with both resistance exercise and aerobic exercise as major components. Resistance exercise includes reports that utilized an acute session or training program of resistance exercise without any significant aerobic exercise component.

The participant population(s) was/were categorized using descriptions within report methods and results sections. "Athlete Participants" includes those reports which specifically mention recruiting professional or recreational athletes as participants, as well as those that included participants pre-screened for high cardiorespiratory fitness. "Healthy Active Participants" includes those reports which mention recruiting healthy active participants. "Inactive Participants and/or Those with Disease" includes those reports which specifically mention recruiting inactive/sedentary participants and/or participants diagnosed with a pathological condition (including obesity).

The cell population studied within a report was determined following review of report methods. "Mixed Cell Populations" includes those reports which specify obtaining data from samples with mixed populations of cells including white blood cells/leukocytes, peripheral blood mononuclear cells (PBMCs), and lymphocytes. "Isolated Cell Populations" includes those reports which specify obtaining data in specific cell types either via physical isolation (e.g., positive or negative selection) or by cell type-specific gating using flow cytometry.

Outcome measures were also determined from report methods and results sections and categorized as listed in section II-1. Additionally, given the terminology associated with real-time assessments of metabolic function, a summary of this terminology is provided to aid comprehension.

Cell metabolic function assessments provided by the Seahorse Analyzer platform include estimates of glycolytic flux (derived from either extracellular acidification rate (ECAR) or proton efflux rate (PER)) and evidence of mitochondrial respiration via oxygen consumption rate (OCR). Introduction of stimulants (e.g., glucose) and inhibitors (e.g., 2-deoxyglucose, oligomycin) is used to test the function of each pathway, and deliver parameters including basal PER/OCR, maximal PER/OCR, reserve PER/spare respiratory capacity, as well as background PER/OCR (e.g., non-mitochondrial OCR). Additional information on the Seahorse platform can be found in (70) and (5).

Metabolic function assessments provided by the Oroboros-O2K platform yield similar information as the Seahorse platform. However, differences in the approach mean that outcome measures are not equivalent. In intact cells, ATP-linked (i.e., OCR associated with production of ATP), uncoupled, maximal & reserve, and non-mitochondrial OCR can be determined following the sequential addition of respiratory chain inhibitors and uncouplers (e.g., Carbonyl cyanide-p-trifluoromethox yphenylhydrazone). Additional information about electron transport system function can be acquired by monitoring OCR of permeabilized cells in the absence and presence of substrates. This approach provides outputs including routine (without and with complex I substrates), leak (without and with complex 2 substrates), complex I-linked, complex II-linked, maximal oxidative phosphorylation (OXPHOS), and maximal electron transport system capacity (ETS) OCR. Further information on the O2k platform and its use can be found in (55) and (19).With the aforementioned caveat that methodological differences mean outputs from the two platforms are not the equivalent, the following outputs from Seahorse and O2k, respectively, provide similar information: basal OCR and routine OCR, proton leak OCR and leak OCR (both), ATP-linked OCR (both), Maximal OCR and ETS, Reserve OCR (both), non-mitochondrial OCR (both). The platform used to measure cellular respiration is indicated throughout the results.

When available, statistics regarding the magnitude of observed effects (effect size, percent change, fold change) are reported following the associated results. However, these data are not included universally as they were infrequently reported throughout the included reports.

RESULTS

Search Results

The database search returned 2363 reports: 548 from Pubmed, 764 from Scopus, and 1051 from Embase. After filtering these results, 52 reports remained. These reports, along with eight additional reports identified via references included in the latter 52, were reviewed in full to confirm their eligibility.

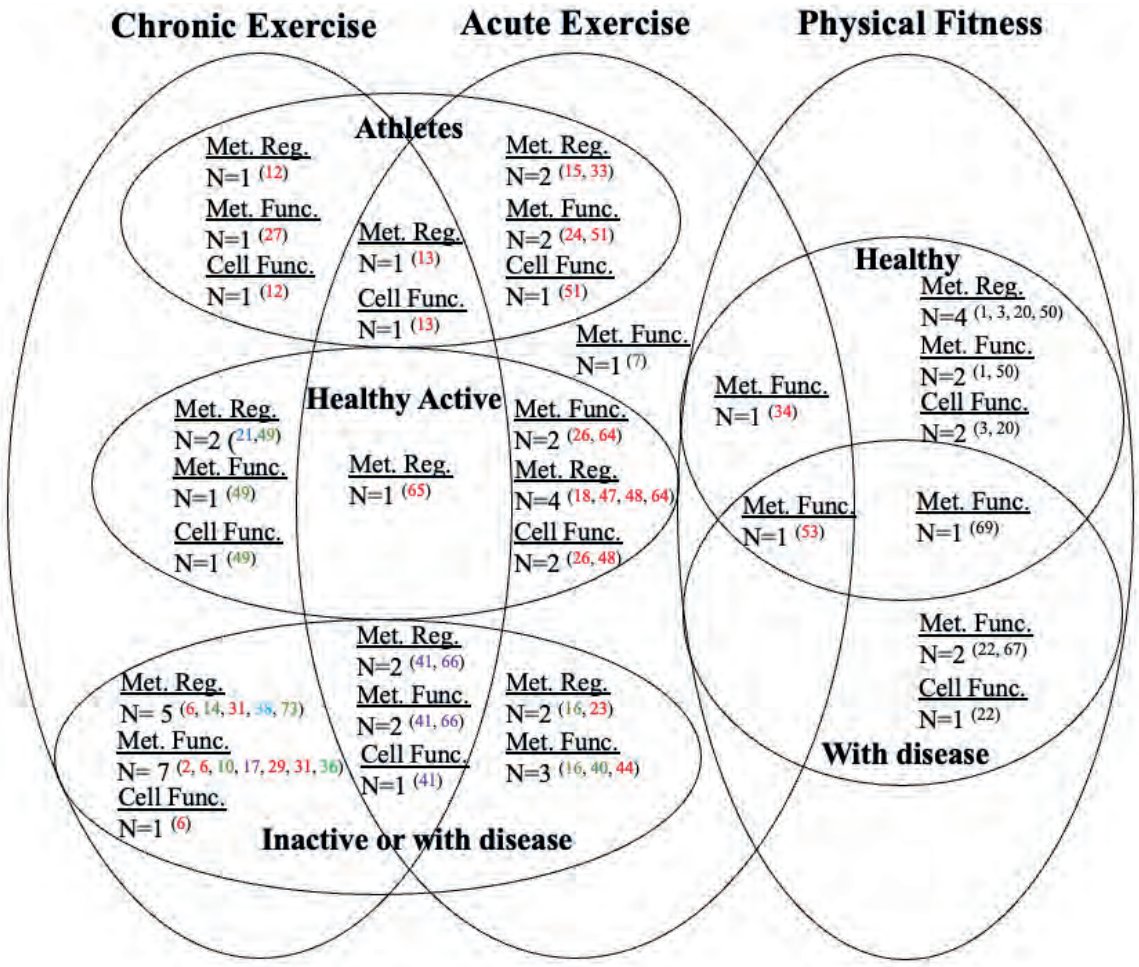


Figure 2:
Summary of experimental designs of retrieved studies examining the impacts of acute exercise, exercise training, and/or physical fitness on human leukocyte metabolic regulation (met. reg.), metabolic function (met. func.), and cell function (cell func.). Large ovals indicate whether effects of chronic or acute exercise or physical fitness were examined; areas of overlap indicate studies with a combined design (e.g., acute and chronic exercise). Smaller ovals indicate population examined; areas of overlap indicate multiple populations examined and studies appearing outside smaller ovals did not specify subject population. Number of articles investigating each of the outcome groups (met. reg., met. func., cell func.) are indicated, along with reference number. The color of the reference number indicates exercise stimulus: red: heavy-severe aerobic exercise; green: light-moderate aerobic exercise; purple: both heavy-severe and light-moderate aerobic exercise; blue: resistance or concurrent exercise.

Nineteen of the 60 reports were excluded following full-text review, leaving 41 reports for inclusion. Figure 1 relates the search and study selection process in detail.

Study Characteristics

Reports varied widely in publication date: the earliest report was published in 1958 and the latest (n=3) were published in 2022. The majority of reports were published since 2014. Most reports included study of inactive adults with or without the presence of disease (n=25), followed by those that included athletes (n=13), and then healthy active adults not formally training (n=9). In three reports the health and/or activity status of some participants could not be determined based on the methods described (3, 7, 20). Leukocyte metabolic function (e.g., cellular respiration) (n=13) was the most common study outcome, while the combination of metabolic regulation (e.g., gene expression), metabolic function, and cell function (e.g., cytolytic protein production) (n=3) was the least common.

Fourteen reports studied the effects of acute exercise on leukocyte energy metabolism (Table 2). Ten used aerobic exercise bouts of heavy-severe intensity including: three graded exercise tests to exhaustion (GXT) (15, 24, 44), four sessions of continuous cycling exercise (18, 47, 48, 64), one session of high intensity intervals (26), one session of continuous cycling ending with a time trial (33), and one pure cycling time trial (51). Four reports implemented aerobic exercise of light-moderate intensity including two bouts of treadmill walking (7, 23) and two bouts of continuous cycling (16, 40). It should be noted that the moderate exercise session in one of the latter four reports was conducted under hypoxic conditions (16).

Fourteen reports investigated the effects of chronic exercise training (i.e., >10-day exercise program) (Table 3). Seven of these reports implemented aerobic exercise of heavy-severe intensity including four programs of high-intensity interval training (HIIT) (2, 6, 17, 31), one program of sprint interval training in hypoxia (27), one program of treadmill exercise (29), and one program of sport-specific training (12). Six reports evaluated the effects of aerobic exercise training of light-moderate intensity. These included two programs of treadmill walking (14, 73), two programs of mixed aerobic exercise (10, 36), one program of continuous cycling (17),

and one trekking program (49). Additionally, one report used concurrent training (38) and one report implemented resistance training (21).

Seven reports evaluated the effects of physical fitness on leukocyte energy metabolism (1, 3, 20, 22, 50, 67, 69). Characteristics of these reports are related in Table 4.

Six reports examined the combination of acute exercise and physical fitness or exercise training on leukocyte energy metabolism (Table 5). Two reports evaluated the effects of acute heavy-severe intensity aerobic exercise amongst individuals of different levels of physical fitness (34, 53), while four reports evaluated effects of both acute and chronic exercise (i.e., acute exercise before and/or after training). Three of these studied the effects of acute aerobic exercise of heavy-severe intensity including one GXT (41), one continuous cycling bout (65), and one session of sport-specific training (13). One report studied the effects of acute cycling exercise of light-moderate intensity under hypoxic conditions (66). Regarding exercise training, three of the reports included at least one study arm of aerobic exercise training of heavy-severe intensity including: two HIIT programs (41, 66), one program of continuous and HIIT cycling (65), and one program of sport-specific training (13). Finally, two of the reports included aerobic exercise training of light-moderate intensity (41, 66). Both reports used programs of continuous cycling.

Key Findings

Effects of Acute Exercise

Key findings obtained from reports on the effects of acute exercise are related in the following sections and in Table 6. These include findings from reports strictly investigating acute exercise, and acute exercise results from those investigating both chronic and acute exercise or physical fitness and acute exercise. For reports on both acute and chronic exercise, results related in this section are restricted to those obtained prior to training or at a single timepoint following training (i.e., no pre-training acute exercise). Unless otherwise noted, data relate to pre-exercise vs. immediately post-exercise changes.

HEAVY-SEVERE INTENSITY AEROBIC EXERCISE: ATHLETE PARTICIPANTS

Mixed Cell Populations

Metabolic Regulation: Three reports investigated the effects of acute aerobic exercise of heavy-severe intensity on metabolic regulation of PBMCs among athletes. Busquets-Cortes et al. evaluated the effects of an acute bout of fitness testing and sport training following eight weeks of training and competition among professional athletes (football/soccer players) (13). No acute exercise testing was performed prior to training. In PBMCs obtained two hours following the acute bout, the authors observed increases in cytochrome C oxidase subunit IV (COXIV), peroxisome proliferator-activated receptor α coactivator 1-alpha (PGC-1α), and mitochondrial NADH dehydrogenase subunit 5 mRNA, as well as PGC-1α, mitochondrial uncoupling protein 2, and mitofusin-2 protein levels (13). Similarly, in trained cyclists, Hunter et al. observed decreased PGC-1α DNA methylation and increased PGC-1α mRNA expression in PBMCs obtained immediately following a bout of cycling exercise (33). In contrast, Capo et al. observed no change in PGC-1α, peroxisome proliferator-activated receptor γ (PPARγ), mitochondrial transcription factor A (Tfam), or mitochondrial uncoupling protein 3 mRNA, but a significant increase in mitochondrial NAD-dependent protein deacetylase sirtuin 3 (SIRT3) mRNA in PBMCs obtained two hours following GXT to exhaustion among taekwondo athletes (15).

Metabolic Function: The effects of acute aerobic exercise of heavy-severe intensity on metabolic function of mixed cell populations were investigated in three reports among athletes. In PBMCs obtained from endurance athletes immediately following GXT, Ferry et al. observed (mean ± standard deviation (SD)) 33.6 ± 6.6% decreased pyruvate dehydrogenase activity, 43.1 ± 4.7% increased citrate synthase activity, and no change in cytochrome C oxidase or succinate cytochrome reductase activities versus pre-exercise (24). Pendergast et al. also observed no change in total fatty acid (FA) oxidation in leukocytes obtained following GXT in elite runners (53). In this study, FA oxidation was determined in whole blood via oxidation of radiolabeled palmitic acid. However, FA oxidation per cell was decreased, ostensibly due to increased cell numbers. Finally, no changes in PBMC respiratory or glycolytic function (assessed via extracellular flux analyses, Seahorse Assay) were observed following one hour of cycling among trained female participants (34). However, it should be noted that blood samples were taken 21 hours post-exercise in that latter report.

Cell Function: One report assessed cell function of mixed cell populations following acute aerobic exercise of heavy-severe intensity among athletes. Intracellular ROS, and phorbol 12-myristate 13-acetate (PMA; 10ng/mL, one hour)-stimulated H_2O_2 production increased versus pre-exercise in PBMCs obtained two hours following exercise in the previously mentioned report of professional soccer players (13). In addition, increased nuclear factor kappa B (NF-κB) activation was also observed in this report. These changes in cell function occurred alongside increased gene and protein expression for markers of mitochondrial respiration and mitochondrial dynamics.

Isolated Cell Populations

Metabolic Regulation: No reports investigated the effects of acute aerobic exercise of heavy-severe intensity on metabolic regulation in isolated cell populations among athletes.

Metabolic Function: One report was retrieved on the effects of acute aerobic exercise of heavy-severe intensity on metabolic function of isolated cell populations among athletes. These authors investigated the effects of an acute 75 km cycling time trial on circulating immunomodulating factors via incubation (six hours) of THP-1 cells with athlete plasma obtained pre-exercise and various timepoints post-exercise (51). In extracellular flux experiments (Seahorse Assay), the authors observed lower OCR (measure of mitochondrial respiration) and greater ECAR (measure of glycolysis) of lipopolysaccharide (LPS)-stimulated (10 ng/mL) THP-1 cells incubated with plasma of athletes consuming only water versus those provide food during exercise (51). Spare respiratory capacity (SRC) of cells incubated with water-only plasma was also lower than the other conditions.

Cell Function: In the aforementioned study no change in

cyclooxygenase-2 mRNA expression was observed versus pre-exercise in THP-1 cells incubated (6 hours) with plasma from immediately, 1.5 hours, or 21 hours post-exercise in the water-only condition (51). Among cells incubated with 21 hours post-exercise plasma, greater cyclooxygenase-2 mRNA expression was observed in cells incubated with plasma from the water-only condition versus food conditions.

HEAVY-SEVERE INTENSITY AEROBIC EXERCISE: HEALTHY ACTIVE PARTICIPANTS

Mixed Cell Populations

Metabolic Regulation: Three reports investigated the effects of acute aerobic exercise of heavy-severe intensity on metabolic regulation of mixed cell populations among healthy active participants. Davies et al. observed elevated PGC-1α, PPARγ, and ATP-binding cassette subfamily A, member 1 (ABCA1) mRNA expression in PBMCs obtained three hours following 45 minutes cycling at 70% VO_{2max} (18). However, at the protein level, there was no significant change observed in phosphorylated AMP-activated protein kinase (AMPK). Elevated fatty acid translocase (CD36) and ABCA1 mRNA expression were also observed in PBMCs obtained at the three hour timepoint following the same prescribed exercise in a separate report (65). In contrast, Moir et al. reported decreased PGC-1α mRNA and AMPK phosphorylation in PBMCs obtained immediately following 45 minutes of cycling exercise at 70% VO_{2max} (48). Both PGC-1α mRNA and AMPK phosphorylation returned to pre-exercise levels by 1-hour post-exercise.

Metabolic Function: Two reports studied metabolic function of mixed cell populations following acute aerobic exercise of heavy-severe intensity among healthy active participants. Frisina et al. documented 18.69% increased glutamine oxidation (measured via liquid scintillation) and 27.02% increased lactate production (measured spectrophotometrically) of lymphocytes obtained three minutes following a bout of 25 one-minute high-intensity treadmill intervals (26). Furthermore, glutamine oxidation was positively correlated with percent change in NK cells ($CD56^+$) pre- to post-exercise ($r = 0.78$, $p < 0.01$), while glutamine oxidation and lactate production were negatively correlated with percent change in T cells (CD3+) pre- to post-exercise ($r = -0.93$, $p < 0.001$, $r = -0.66$, $p < 0.01$). Theall et al. observed no significant change pre- versus immediately post-exercise (30 min. at 65-75% VO_{2peak}) in PBMC routine or leak respiration, OXPHOS, or ETS per million cells as assessed by high resolution respirometry (Oxygraph) (64). However, pre- to post-exercise increases in routine ($d = 0.58$) and leak (in presence of pyruvate + malate + glutamate + succinate only) ($d = 0.80$) respiration, OXPHOS ($d = 0.80$), and ETS ($d = 0.78$) were observed when quantified per milliliter of blood.

Cell Function: Two reports studied the effects of acute aerobic exercise of heavy-severe intensity on cell function of mixed cell populations among healthy active participants. Decreased (58%) lymphocyte proliferative responses to concanavalin A (Con-A) (3.5 μg/mL, 48 hrs.) were observed following interval exercise in the report by Frisina et al. Additionally, pre- to post-exercise changes in lymphocyte proliferation positively correlated with percent change in T cells ($CD3^+$) ($r = 0.78$, $p < 0.01$) and negatively correlated with percent change in NK cells ($CD56^+$) ($r = -0.76$, $p < 0.05$) (26). The authors also report correlations between exercise induced changes in lymphocyte proliferative responses and glutamine oxidation, and lactate production. However, the directions of these relationships were not disclosed. Finally, in a separate report, decreased basal and PMA-stimulated (25 ng/mL, 10 minutes) ROS production was observed in PBMCs obtained immediately following 45 minutes cycling at 70% VO_{2max} (48). For context, decreased PGC-1α mRNA expression and AMPK phosphorylation were also observed at this timepoint in the same report.

Isolated Cell Populations

Metabolic Regulation: Two reports assessed the effects of acute aerobic exercise of heavy-severe intensity on metabolic regulation of isolated cell populations among healthy active participants. Monocytes (isolated from PBMCs via immunomagnetic separation) obtained from healthy active male participants immediately post-45 minutes of cycling exercise at 70% VO_{2max} demonstrated no change in CD36 mRNA expression but decreased AMPK phosphorylation versus pre-exercise (47). However, monocyte AMPK phosphorylation returned to baseline and CD36 remained stable one-hour post-exercise in the same report. Cycling exercise (30 minutes 65-75% VO_{2max}) was also demonstrated to alter nutrient transport and metabolism proteins of peripheral T cells. Compared to pre-exercise, increased numbers of glucose transporter type 4 positive ($GLUT4^+$) cells (+53%), but decreased proportions of hexokinase 2 positive ($HK2^+$) and numbers (-55%) and proportions of hexokinase 1 positive ($HK1^+$) CD4+ T cells were observed within post-exercise PBMCs in the report from Theall et al. (64). The authors also observed decreases in the proportions of CD36+ and HK1+ CD8+ T cells within PBMCs pre- to post-exercise. Finally, exercise-associated changes in nutrient transport and metabolism proteins were correlated with expression of a number of cell activation markers (Table 6).

Metabolic Function, Cell Function: No reports investigated the effects of acute aerobic exercise of heavy-severe intensity on metabolic function or cell function in isolated cell populations among healthy active participants.

HEAVY-SEVERE INTENSITY AEROBIC EXERCISE: INACTIVE PARTICIPANTS AND/OR THOSE WITH DISEASE

Mixed Cell Populations

Metabolic Regulation: No reports investigated the effects of acute aerobic exercise of heavy-severe intensity on metabolic regulation in mixed cell populations among inactive participants and/or those with disease.

Metabolic Function: The effects of acute aerobic exercise of heavy-severe intensity on metabolic function of mixed cell populations among inactive participants and/or those with disease were investigated in three reports. No change in PBMC respiratory or glycolytic function was observed following a one hour bout of cycling at 70% VO_{2peak} among inactive female participants in the report from Janssen et al. (34). It should be noted that post-exercise blood samples were obtained 21 hours following the original bout. Meksawan et al. and Pendergast et

al. evaluated the effects of acute treadmill GXT on leukocyte FA oxidation (whole blood oxidation of radiolabeled palmitic acid) among sedentary participants, or those with underlying pathology (44, 53). Meksawan et al. observed an increase in leukocyte FA oxidation per milliliter of blood, but a decrease when quantified on a per cell basis. Pendergast et al. also observed an increase in total leukocyte FA oxidation but no change in FA oxidation per cell among their inactive participants.

Cell Function: No reports investigated the effects of acute aerobic exercise of heavy-severe intensity on cell function, in relation to cellular metabolism, in mixed cell populations among inactive participants and/or those with disease.

Isolated Cell Populations
Metabolic Regulation: Lin et al. authored the sole report to evaluate the effects of acute heavy-severe exercise on metabolic regulation of isolated cell populations. NK cells were isolated from PBMCs via negative-immunomagnetic selection among inactive participants and/or those with disease. They observed no change in NK cell mitochondrial content or mitochondrial membrane potential (MMP), but an increase in mitochondrial oxidant burden pre- vs. post-exercise (cycle ergometer GXT) among sedentary male participants (41).

Metabolic Function: Lin et al. were also the only report to assess the effects of acute heavy-severe exercise on metabolic function of isolated cell populations among inactive participants and/or those with disease. Measurements of mitochondrial respiration were made via Oxygraph. Increases in ETS, Reserve OCR, and the bioenergetic health index (BHI) were observed in NK cells obtained immediately post-exercise versus pre-exercise (41).

Cell Function: Elevated NK cell perforin and granzyme b expression were observed immediately post- versus pre-exercise in the report from Lin et al. (41). NK cells were unstimulated in this study.

LIGHT-MODERATE INTENSITY AEROBIC EXERCISE: ATHLETE PARTICIPANTS
No reports investigated the effects of acute aerobic exercise of light-moderate intensity on cellular metabolism and function in peripheral leukocytes among athletes.

LIGHT-MODERATE INTENSITY AEROBIC EXERCISE: HEALTHY ACTIVE PARTICIPANTS
No reports investigated the effects of acute aerobic exercise of light-moderate intensity on cellular metabolism and function in peripheral leukocytes among healthy active participants.

LIGHT-MODERATE INTENSITY AEROBIC EXERCISE: INACTIVE PARTICIPANTS AND/OR THOSE WITH DISEASE

Mixed Cell Populations
Metabolic Regulation: The effects of acute aerobic exercise of light-moderate intensity on metabolic regulation of mixed cell populations were assessed in three reports. As a part of a larger investigation, Ferrer et al. instituted a 30-minute bout of treadmill walking (60-70% maximum heart rate) in older men with obesity and metabolic syndrome (23). They observed decreased COXIV and Tfam mRNA expression, and no significant change in mitochondrial respiration or mitochondrial dynamics protein levels in PBMCs obtained 30 minutes post-exercise. Similarly, Tsai et al. observed no significant changes in mitochondrial biogenesis and mitochondrial dynamics protein levels, or mitochondrial count of lymphocytes obtained following 36 minutes cycling exercise among young sedentary males (66). However, MMP was decreased and mitochondrial oxidant burden was increased following exercise. Though the absolute and relative workload of the latter bout was low (100 W, ~50% maximum Watts), it must be noted that the exercise was performed under hypoxic conditions (12% O2) (66). This undoubtedly increased the difficulty of the bout and resulting physiological responses. In contrast to Tsai et al., a separate report observed increased MMP in PBMCs obtained immediately following 40 minutes cycling exercise in hypoxia (60% $VO_{2ma}x$, 12% O2) (16).

Metabolic Function: In the same report discussed immediately previous (16), no differences in routine or maximal mitochondrial OCR measured by Oxygraph were observed post-exercise versus pre-exercise. However, the authors did report depressed OCR specifically via respiratory chain complex I (CI) in permeabilized cells. Depressed CI OCR (also measured by Oxygraph) post-exercise was also observed in permeabilized lymphocytes following exercise in hypoxia in the report from Tsai et al. (66). Additionally, these authors noted decreased OCR through complex II (CII) in permeabilized cells, decreased ATP-linked and Reserve OCR in intact cells, increased lactate dehydrogenase and glutamate dehydrogenase enzyme activity, and decreased citrate synthase activity post- versus pre-exercise (66). Depressed metabolic function following exercise is not a universal finding in these settings (light-moderate exercise, inactive/disease-burdened participants), however. Among sedentary men and women, Liepinsh et al. investigated respiratory function of PBMCs obtained 15 minutes following a one-hour bout of low-intensity (50 W, ~36% VO_{2max}) cycling exercise via Oxygraph (40). These authors observed increased routine OCR (+31%) in intact cells, as well as increased FA-dependent leak OCR (+65%), OXPHOS (+76%), and oxidative phosphorylation coupling efficiency (+22%) in permeabilized cells.

Cell Function: No reports investigated the effects of acute aerobic exercise of light-moderate intensity on cell function, in relation to cellular metabolism, in mixed cell populations among inactive participants and/or those with disease.

Isolated Cell Populations
No reports investigated the effects of acute aerobic exercise of light-moderate intensity on cellular metabolism and function in isolated cell populations among inactive participants and/or those with disease.

LIGHT-MODERATE INTENSITY AEROBIC EXERCISE: UNCATEGORIZED PARTICIPANTS

Bisset et al. investigated the effects of a short bout (3-4 minutes) of treadmill exercise on leukocyte respiration using a Warburg Respirometer (7). The exercise bout elicited an increase in systemic VO_2 (i.e., of the participants) of 8-49% versus pre-

exercise. In cells obtained 5 minutes post-exercise, the authors observed a 36-71% increase in leukocyte oxygen consumption. The health and activity characteristics of the participants related in the report were not clear, precluding aggregation with other reports of acute exercise.

Concurrent Exercise
No reports investigated the effects of acute exercise including both aerobic and resistance components on cellular metabolism and function in peripheral leukocytes.

Resistance Exercise
No reports investigated the effects of acute resistance exercise on cellular metabolism and function in peripheral leukocytes.

Effects of Chronic Exercise
Key findings regarding the effects of chronic exercise training on peripheral leukocyte metabolism and function are related in the following sections and in Table 7. These include results from both reports strictly investigating chronic exercise, as well as chronic exercise data from reports on the effects of both acute and chronic exercise. Unless otherwise noted, key findings refer to changes observed pre- to post-training in resting participants.

HEAVY-SEVERE INTENSITY AEROBIC EXERCISE TRAINING: ATHLETE PARTICIPANTS

Mixed Cell Populations
Metabolic Regulation: Two reports from the same author group investigated the effects of eight weeks of sport-specific training on PBMC metabolic regulation among male athletes (football/soccer) (12, 13). The authors observed increased Tfam, OPA1 dynamin like GTPase, and OMA1 zinc metallopeptidase protein expression in the first report (12). In the later report, increased mitochondrial uncoupling protein 2, mitochondrial uncoupling protein 3, COXIV and mitofusin 1, but decreased Tfam protein expression were observed (13). Additionally, no change in COXIV, PGC-1α, or mitochondrial NADH dehydrogenase subunit 5 mRNA expression was noted in this later report.

Metabolic Function: One study evaluated the effects of heavy-severe intensity training on metabolic function of mixed cell populations among athletes (27). Following three weeks of hypoxic sprint interval (SIH) or repeated sprint (RSH) cycling training in recreational athletes, the authors observed diminished PBMC ETS (RSH only) and PBMC ETS/citrate synthase activity (RSH & SIH) via Oxygraph. These results were obtained from only two participants per group and should be interpreted accordingly.

Cell Function: The two reports which investigated the effects of heavy-severe intensity aerobic exercise training on metabolic regulation also assessed the effects of training on cell function of PBMCs. In the earlier of the two reports, elevated PBMC protein carbonyls but decreased malondialdehyde levels were noted following the eight weeks of training (12). This suggests training altered redox balance in the cells. However, no change was observed in PMA-stimulated (10 ng/mL) H_2O_2 production. No change in PMA-stimulated (10 ng/mL) H_2O_2 production was also noted following training in the later report, as well as no difference in NF-κB activation versus pre-training (13).

Isolated Cell Populations
No reports investigated the effects of aerobic exercise training of heavy-severe intensity on cellular metabolism and function in isolated cell populations among athletes.

HEAVY-SEVERE INTENSITY AEROBIC EXERCISE TRAINING: HEALTHY ACTIVE PARTICIPANTS

Mixed Cell Populations
Metabolic Regulation: In the sole report of the effects of heavy-severe aerobic exercise training on metabolic regulation of mixed leukocyte populations in health active adults, Thomas et al. observed increased PBMC CD36 protein expression following eight weeks of continuous and interval-based cycling training (65). However, no significant change in PPARγ protein expression or in PPARγ phosphorylation was observed.

Metabolic Function, Cell Function: No reports investigated the effects of aerobic exercise training of heavy-severe intensity on metabolic function or cell function in mixed cell populations among healthy active participants.

Isolated Cell Populations
No reports investigated the effects of aerobic exercise training of heavy-severe intensity on cellular metabolism and function in isolated cell populations among healthy active participants.

HEAVY-SEVERE INTENSITY AEROBIC EXERCISE TRAINING: INACTIVE PARTICIPANTS AND/OR THOSE WITH DISEASE

Mixed Cell Populations
Metabolic Regulation: Two reports studied the effects of heavy-severe aerobic exercise training on metabolic regulation of mixed cell populations among inactive and/or participants with disease. Both reports observed minimal change in metabolic regulation of mixed leukocyte populations following training in inactive adults. After two weeks of HIIT by cycle ergometer, Hedges et al. noted no changes in PBMC PGC-1α, Tfam, nuclear respiratory factor 1, nuclear respiratory factor 2, or COXIV mRNA, or electron transport chain CI-CV protein expression (31). Similarly, six weeks of cycling HIIT did not change lymphocyte mitochondrial biogenesis proteins in the report from Tsai et al. (66). However, the latter authors did note declines in mitofusin and dynamin-related protein-1 (DRP-1). The greater relative decline in DRP-1 led to an increase in mitofusin:DRP-1 post-training versus pre-training, suggestive of increased mitochondrial fusion versus fission (66).

Metabolic Function: Four reports related data regarding the effects of heavy-severe aerobic exercise training on metabolic function in mixed cell populations in inactive individuals and/or those with disease. Three of these reports recruited sedentary but otherwise healthy males (17, 31, 66), and one report detailed the effects of exercise in women with systemic lupus erythematosus (23). Using Oxygraph, Chang and Wang (2015) observed increased ATP-linked OCR and decreased non-mitochondrial OCR in intact lymphocytes, and increased CI-linked OCR following six weeks of cycling HIIT (17). Tsai et al. also observed via Oxygraph increased ATP-linked OCR and Reserve OCR in intact lymphocytes following six

weeks cycling HIIT, as well as increased CII-linked OCR and OXPHOS of permeabilized cells, and increased succinate dehydrogenase activity (66). These authors also assessed the effects of acute hypoxic exercise (36 min, 100 W, 12% O2) following training and observed smaller exercise-induced depressions in lymphocyte OCR, and lactate dehydrogenase, succinate dehydrogenase, and citrate synthase activity (66). Contrary to the previous two reports, Hedges et al. noted no change in PBMC mitochondrial respiration (measured by Oxygraph) following two weeks HIIT despite improvements in mitochondrial respiration of permeabilized skeletal muscle fibers (31). Accordingly, skeletal muscle mitochondrial respiration was not correlated with PBMC respiration either pre- or post-training. Finally, Hasni et al. found an improvement in PBMC OCR:ECAR measured by Seahorse assay following 12 weeks treadmill exercise in women with systemic lupus erythematosus (29). Additionally, the change in OCR:ECAR was negatively correlated with change in self-reported fatigue (via Fatigue Severity Scale) ($r = -0.59$, $p = 0.03$) over the intervention (i.e., increase OCR:ECAR associated with decreased fatigue).

Cell Function: No reports investigated the effects of aerobic exercise training of heavy-severe intensity on cell function, in relation to cellular metabolism, in mixed cell populations among inactive participants and/or those with disease.

Isolated Cell Populations

Metabolic Regulation: Two reports presented data on the effects of heavy-severe aerobic exercise training on metabolic regulation in isolated cell populations in inactive individuals and/or those with disease. Bartlett et al. observed increased neutrophil MMP ($d = 1.10$) following ten weeks of treadmill HIIT in prediabetic overweight-obese older adults (6). Likewise, Lin et al. noted increased NK cell MMP at rest following six weeks of cycling HIIT in sedentary young males (41). The latter authors also observed smaller exercise-induced perturbations to NK cell MMP and mitochondrial oxidant burden (e.g., mitochondrial reactive oxygen species) in response to cycling GXT post-training (41).

Metabolic Function: The effects of heavy-severe intensity aerobic exercise training on metabolic function of isolated cell populations among inactive participants and/or those with disease were investigated in three reports. Following ten weeks of HIIT among adults afflicted with rheumatoid arthritis, Andonian et al. found via Seahorse assay that improvements in cardiorespiratory fitness positively correlated with post-training basal and maximal respiration of $CD4^+$ T cells ($\rho = 0.89$, $p = 0.019$ for both), and that improvements to CD4+ T cell mitochondrial respiration positively correlated with increases in CD4+ CCR7+CD45RA+ T cells (i.e., naïve cells) ($\rho = 0.89$, $p = 0.019$) (2). Among the cohort of prediabetic overweight-obese older adults discussed in the previous section, increased neutrophil basal respiration ($d = 3.01$), maximal respiration ($d = 2.86$), and ATP production ($d = 4.18$) were observed following training (measured by Seahorse assay) (6). Finally, using Oxygraph, Lin et al. noted increased NK cell ETS, Reserve OCR, and BHI at rest following six weeks HIIT in sedentary young male participants (41). These authors also found that the change in VO_{2max} pre- to post-training was positively correlated with pre- to post-training changes in ETS ($r = 0.549$, $p < 0.001$), Reserve OCR ($r = 0.655$, $p < 0.001$), and BHI ($r = 0.546$, $p < 0.001$).

Cell Function: Bartlett et al. and Lin et al. were the only groups to investigate the effects of heavy-severe intensity aerobic exercise training on cell function of isolated cell populations, in relation to cellular metabolism, among inactive participants and/or those with disease. The former group found improvements in a variety of measures of neutrophil function following training (Table 7), in parallel to the metabolic changes described in the previous sections (6). Notably, pre- to post-training changes in neutrophil chemotaxis were positively correlated with percent change in VO_{2peak} ($r = 0.649$, $p = 0.042$) and negatively correlated with change in relative body fat ($r = -0.721$, $p = 0.018$). These relationships suggest integration between systemic and cellular effects of HIIT training. In NK cells, Lin et al. noted increased perforin and granzyme b expression in unstimulated NK cells obtained from resting participants post-training (41). These functional changes complimented the training-related improvements in mitochondrial regulation and respiratory function previously described.

LIGHT-MODERATE INTENSITY AEROBIC EXERCISE TRAINING: ATHLETE PARTICIPANTS

No reports investigated the effects of aerobic exercise training of light-moderate intensity on cellular metabolism and function in peripheral leukocytes among athletes.

LIGHT-MODERATE INTENSITY AEROBIC EXERCISE TRAINING: HEALTHY ACTIVE PARTICIPANTS

Mixed Cell Populations

Metabolic Regulation: Only Morabito et al. investigated the effects of light-moderate aerobic exercise training on metabolic regulation of mixed cell populations in healthy active adults. These authors observed no changes in hypoxia inducible factor 1 subunit alpha (HIF-1α) mRNA expression or MMP of PBMCs obtained following ten days trekking at either low- (mean ± standard error of the mean (SEM): 598 ± 561m) or high-altitudes (4132 ± 863m) in the same participants (49). However, decreased mitochondrial ROS was noted following trekking at high-altitude.

Metabolic Function: In the same experiment discussed in the previous section, elevated PBMC metabolic activity (via staining with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT)) was observed following high-altitude but not low-altitude trekking (49).

Cell Function: Morabito et al. observed no change in unstimulated PBMC intracellular ROS or nitric oxide following either low- or high-altitude trekking (49).

Isolated Cell Populations

No reports investigated the effects of aerobic exercise training of light-moderate intensity on cellular metabolism and function in isolated cell populations among healthy active participants.

LIGHT-MODERATE INTENSITY AEROBIC EXERCISE TRAINING: INACTIVE PARTICIPANTS AND/OR

THOSE WITH DISEASE

Mixed Cell Populations

Metabolic Regulation: The effects of light-moderate aerobic exercise training on metabolic regulation of mixed leukocyte populations among inactive participants and/or those with disease were investigated in three reports. Implementing eight-week treadmill walking program among middle-aged (mean ± SD: 45.6 ± 11.1 years) adults, Butcher et al. observed increases in PBMC CD36 (mean ± SD: 3.86 ± 0.61-fold, 2.72 ± 0.53-fold) and PPAR (1.82 ± 0.89-fold, 4.27 ± 1.89-fold) mRNA expression at four and eight weeks of training versus pre-training or non-training participants (i.e., controls) (14). Increases in PBMC ABCA1 (3.46 ± 0.56-fold) and ATP-binding cassette subfamily G member 1 (ABCG1) (3.06 ± 0.47-fold) mRNA expression were also observed at eight weeks compared to pre-training or control participants. Similarly, Yakeu et al. noted increased PBMC PGC-1α (4-fold, 5-fold) and peroxisome proliferator-activated receptor α (PPARα) (1.6-fold, 1.6-fold) mRNA expression after four and eight weeks of training, and increased peroxisome proliferator-activated receptor γ coactivator 1-beta (PGC-1β) (2-fold) mRNA expression solely after eight weeks of treadmill training (73). Similar to HIIT, Tsai et al. found no change in lymphocyte mitochondrial biogenesis protein levels, and a decrease in mitofusin and DRP-1 following six weeks cycling moderate intensity continuous training (MICT) at 60% VO_{2max}(66). Unlike HIIT, the ratio of mitofusin to DRP-1 was not significantly altered via MICT (66).

Metabolic Function: Four reports related data on the effects of light-moderate intensity aerobic exercise on metabolic function of mixed cell populations among inactive participants and/or those with disease. Brand et al. observed increased lymphocyte OXPHOS (d = 1.27), ETS (d = 1.67), and CIV (d = 1.08) activity following 12 weeks training in men diagnosed with occupational burnout (10), as well as increased lymphocyte ATP content. Increased PBMC non-mitochondrial respiration (3.15-fold), maximal respiration (2.45-fold) and SRC (5.65-fold) were also observed by Kocher et al. following a twelve week training program in HIV+ men (36). Though, it should be noted the analytic platforms used in these two reports were not the same (Oxygraph-2k versus Seahorse XFe24, respectively). Therefore, the outcomes should not be interpreted as equivalent.

Finally, both Chang and Wang (2015) and Tsai et al. reported on the effects of cycling MICT at 60% VO_{2max} (17, 66). In the earlier of the two reports, Change and Wang observed increased lymphocyte ATP-linked OCR and decreased non-mitochondrial OCR in intact cells, as well as increased CII-linked OCR in permeabilized cells (17). The latter result contrasted with their participants that performed HIIT in whom increased CI-linked OCR was observed rather than CII. Tsai et al. also observed increased lymphocyte ATP-linked and Reserve OCR in intact cells following training, along with increased CII-linked OCR and OXPHOS in permeabilized cells, and increased succinate dehydrogenase activity (66). Finally, Tsai et al. also assessed the effects of acute exercise in hypoxia following training versus prior. Compared to pre-training, these authors observed smaller reductions in lymphocyte respiratory capacity, succinate dehydrogenase activity, as well as lactate dehydrogenase and citrate synthase activity due to hypoxic exercise (66). These trends were the same as those observed among their participants performing cycling HIIT rather than MICT. Both the reports of Chang and Wang and Tsai et al. utilized the same analytic platform (Oxygraph-2K) for measuring metabolic function.

Cell Function: No reports investigated the effects of aerobic exercise training of light-moderate intensity on cell function, in relation to cellular metabolism, in mixed cell populations among inactive participants and/or those with disease.

Isolated Cell Populations

Metabolic Regulation: One report investigated the effects of light-moderate intensity aerobic exercise training on metabolic regulation (as well as metabolic function and cell function) in isolated cell populations among inactive participants and/or those with disease. As they did with their participants performing HIIT, Lin et al. found increased NK cell mitochondrial content and MMP, and decreased mitochondrial oxidant burden following six weeks of cycling MICT at 60% VO_{2max} (41). Notably, GXT post-training also led to smaller reductions in mitochondrial count and membrane potential, as well as smaller increases in mitochondrial oxidant burden versus GXT pre-training.

Metabolic Function: Lin et al. also observed increased ETS, Reserve OCR and BHI of NK cells, and a positive correlation between change in participant VO_{2max} versus change in the latter measures of NK cell respiratory function (ΔVO_{2peak} vs. ETS $r = 0.549$, $p < 0.001$; ΔVO_{2peak} vs. Reserve OCR $r = 0.655$, $p < 0.001$; ΔVO_{2peak} vs. BHI $r = 0.546$, $p < 0.001$) as measured by Oxygraph (41). These trends were the same as those observed among participants in the study performing HIIT rather than MICT.

Cell Function: In the same report discussed in the previous two sections, unstimulated NK cell perforin and granzyme b were found higher post-training versus pre-training and this result was the same as what was observed among participants that completed HIIT (41).

Concurrent Exercise Training: Athlete Participants or Healthy Active Participants

No reports investigated the effects of concurrent exercise training on cellular metabolism and function of peripheral leukocytes among athletes or healthy active participants.

Concurrent Exercise Training: Inactive Participants and/or Those with Disease

Mixed Cell Populations

Metabolic Regulation: Lehti et al. were the sole report on the effects of concurrent exercise training (38). As assessed by microarray, these authors recorded increased PBMC expression of oxidative phosphorylation genes ($z = 3.27$) among those participants that improved VO_{2max} following 21 weeks of training (n=7). All participants, both those included the gene expression sub-study and those not included, were sedentary adults with stable coronary artery disease.

Metabolic Function or Cell Function: No reports investigated the effects of concurrent exercise training on metabolic function or cell function, in relation to cellular metabolism, in mixed cell populations among inactive participants and/or those with disease.

Isolated Cell Populations
No reports investigated the effects of concurrent exercise training on cellular metabolism and function in isolated cell populations among inactive participants and/or those with disease.

RESISTANCE EXERCISE TRAINING: ATHLETE PARTICIPANTS
No reports investigated the effects of resistance training on cellular metabolism and function in peripheral leukocytes among athletes.

RESISTANCE EXERCISE TRAINING: HEALTHY ACTIVE PARTICIPANTS

Mixed Cell Populations
Metabolic Regulation: One report related data on the effects of resistance training on leukocyte metabolic regulation (21). Among healthy active older adults (mean ± SEM: 73.7 ± 2.2 years) randomized to eight weeks of resistance training, increased PBMC PGC-1α and mitofusin 1 protein expression were observed post- versus pre-training. Mitofusin 1 expression post-training among trained participants was also greater than in the control group.

Metabolic Function or Cell Function: No reports investigated the effects of resistance training on metabolic function or cell function in mixed cell populations among healthy active participants.

Isolated Cell Populations
No reports investigated the effects of resistance training on cellular metabolism and function in isolated cell populations among healthy active participants.

RESISTANCE EXERCISE: INACTIVE PARTICIPANTS AND/OR THOSE WITH DISEASE

No reports investigated the effects of resistance training on cellular metabolism and function in peripheral leukocytes among inactive participants and/or those with disease.

Effects of Physical Fitness
Key findings regarding the effects of physical fitness on peripheral leukocyte metabolism and function are related in the following sections and in Table 8. These include results from reports strictly investigating physical fitness, as well as physical fitness data from reports on the effects of both physical fitness and acute exercise. Unless otherwise noted, key findings refer to differences observed between participant fitness classes in samples obtained from resting participants. Categorization by exercise intensity/type and participant population is not performed in this section due to the inclusion of multiple participants populations and a lack of acute or chronic exercise in these reports.

Mixed Cell Populations
Metabolic Regulation: Two reports compared metabolic regulation of mixed cell populations between participants varying in physical fitness. In lymphocytes, Dorneles et al. observed greater mitochondrial membrane depolarization of cells from low-fitness participants (mean ± SD: 38.1 ± 1.7 mL/kg/min. VO_{2peak}) versus cells of those in the moderate-fitness (43.9 ± 2.7 mL/kg/min. VO_{2peak}) and high-fitness groups (55.1 ± 4.7 mL/kg/min.VO_{2peak}) when stimulated with 10 μg/mL Con-A (20). No differences in mitochondrial membrane polarization were observed among unstimulated and 5 μg/mL Con-A stimulated lymphocytes. Mota et al. also noted differences in mitochondrial ROS production in their report comparing men of lower versus higher cardiorespiratory fitness across three age-groups (19-29 years mean ± SD: 42.9 ± 5.1 vs. 57.4 ± 3.9 ml/kg/min. VO_{2max}, 30-39 years 38.5 ± 1.9 vs. 51.3 ± 3.3 mL/kg/min. VO_{2max}, 40-59 years 33.9 ± 5.5 vs. 49.1 ± 5.5 mL/kg/min. VO_{2max}) (50). Compared to the men of higher fitness in each age-group, unstimulated lymphocytes of men in the lower fitness group demonstrated greater mitochondrial H_2O_2 production (50).

Metabolic Function: Metabolic function was the most studied outcome area in reports investigating the effects of physical fitness on mixed cell populations, with six reports retrieved. Vladutiu et al., measuring oxidation of radiolabeled palmitic acid in whole blood, found greater FA oxidation in leukocytes of endurance-trained runners (central tendency not reported: 47.9 mL/kg/min. VO_{2peak}) and sedentary women (34.3 mL/kg/min. VO_{2peak}) versus three female siblings with genetic deficiency for the FA transporter carnitine palmitoyltransferase 2 (CPT2) (36.5, 39.5, and 43.6 mL/kg/min. VO_{2peak}) (69). Greater leukocyte FA oxidation was also observed among the endurance trained versus sedentary women, and a positive correlation was found between leukocyte FA oxidation versus VO_2 achieved at an RER of 1.0 during GXT (r = 0.94, p-value not reported). In a later report also evaluating leukocyte FA oxidation by the same technique, inactive participants (male and female) and non-elite athletes (runners) did not differ, nor did leukocyte FA oxidation differ between inactive participants and those afflicted with a variety of diseases (Table 8) (53). The notable exception in this regard was that of participants with chronic fatigue syndrome, whose values for leukocyte FA oxidation exceeded those of the untrained. Leukocyte FA oxidation of elite athletes (endurance runners) was greater than that of non-elite athletes and the inactive, and FA oxidation of females with genetic deficiency in CPT2 (two of the aforementioned 3 siblings) was less than all other groups (53).

In their comparison of lymphocyte metabolic function across age and fitness, Mota et al. measured lymphocyte oxygen utilization polarographically by a Clarke electrode. The authors report greater CI activity among lower fitness individuals in the 19-29 and 30-39 years age-groups compared to those of higher fitness at each age (50). No difference in CI activity was observed between fitness levels in the 40-59 years age-group. Additionally, a negative correlation was noted between lymphocyte CI activity versus age (i.e., decline in CI activity with advancing age) (r = -0.472, p-value not reported) only among those of lower fitness (50). Tyrell et al. also observed relationships between physical fitness and leukocyte respiratory function (Seahorse assay) in the context of aging (67). Among a cohort of overweight and obese older adults (65-79 years), positive correlations were found between PBMC maximal OCR and SRC versus various measures of physical fitness and body composition (Table 8). A positive correlation was also observed between PBMC basal OCR versus knee extensor maximal

strength ($r = 0.51$, $p < 0.05$), and negative correlations were observed between PBMC basal OCR ($r = -0.61$, $p < 0.05$), maximal OCR ($r = -0.58$, $p < 0.05$), and SRC ($r = -0.55$, $p < 0.05$) versus plasma IL-6 (67). In contrast to the results of the latter two reports, Farinha et al. observed no differences in mitochondrial or non-mitochondrial metabolic activity of PBMCs of healthy active (mean ± SD: 22.22 ± 4.67 mL/kg/min. VO_{2max}) versus inactive (18.6 ± 2.01 mL/kg/min. VO_{2max}) postmenopausal women (22). These conclusions were based on PBMC CI and CII activity, and MTT staining.

Janssen et al. were the only report to assess the effects of physical fitness in both stimulated and unstimulated mixed cells (34). Using the Seahorse Assay, in unstimulated PBMCs, these authors noted greater values for various measures of mitochondrial respiratory function among participants (females only) of high fitness (median: 50.4 mL/kg/min. VO_{2peak}) versus those of low-fitness (median: 35.1 mL/kg/min. VO_{2peak}), but no differences in glycolytic function between groups (34). Stimulation with Con-A (25 μg/mL) increased mitochondrial respiration and glycolysis in both groups. However, Con-A-stimulated basal and maximal OCR of PBMCs from high-fitness participants was greater than those of the low-fitness participants (34). Glycolytic function in Con-A-stimulated PBMCs did not differ between the groups.

Cell Function: Differences in cell function of mixed cell populations relative to physical fitness were reported among both young male and postmenopausal female participants. Among young male participants, Dorneles et al. found that unstimulated and 5 μg/mL Con-A-stimulated lymphocytes from lower-fitness participants produced greater ROS than moderate-fitness and high-fitness participants (20). However, no differences in ROS production were observed between groups when cells were stimulated with 10 μg/mL Con-A (i.e., highest concentration). Unstimulated and 5 μg/mL stimulated lymphocytes from high-fitness participants also demonstrated lower proliferation than cells of the moderate-fitness and low-fitness groups, with no group differences in proliferation observed following stimulation with 10 μg/mL Con-A (20). In contrast to these results, Farinha et al. observed unstimulated ROS production of PBMCs from healthy active postmenopausal women to be greater than that of inactive women (22). However, superoxide dismutase and catalase activity were also greater in cells from healthy active women versus the inactive, and this elevated antioxidant activity may have provided some protection from elevated ROS in the active women.

Isolated Cell Populations

Metabolic Regulation: Antunes et al. and Alley et al. were the only two reports to assess the effects of physical fitness on metabolic regulation of isolated leukocyte populations. In monocytes isolated from PBMCs by tissue-culture adherence, Antunes et al. observed greater LPS-stimulated (100 ng/mL) PPARγ mRNA among participants of high-fitness (mean: 63.1 mL/kg/min. VO_{2max}) versus those in the low-fitness group (35.3 mL/kg/min. VO_{2max}) (3). The authors also noted greater PGC-1α mRNA expression in cells from high-fitness participants under all treatment conditions (LPS 100 ng/mL, rosiglitazone 1 μM, LPS 100 ng/mL + rosiglitazone 1 μM) (group effect $\eta_2 = 0.513$). However, greater AMPK mRNA expression was observed under all treatment conditions in cells of the low-fitness group (group effect $\eta_2 = 0.372$).

Relationships between physical fitness and metabolic regulation in naïve CD8+ and CD4+ T cells were evaluated in the report from Alley et al. (1). In this report, flow cytometric analyses of mitochondria allowed assessment of T cell populations within the PBMC pool. Naïve CD8+ T cells from participants in the high-fitness group (mean ± SD: 59.6 ± 9.0 mL/kg/min. VO_{2peak}) exhibited greater mitochondrial mass than cells of the inactive group (42.7 ± 7.4 mL/kg/min. VO_{2peak}) ($d = 0.76$). However, the groups were not different in MMP and mitochondrial biogenesis of naïve CD8+ or CD4+ T cells. Positive correlations among all participants were noted for both mitochondrial mass of naïve CD4+ and CD8+ T cells versus estimated participant energy expenditure ($r = 0.41$, $p = 0.024$ and $r = 0.36$, $p = 0.048$), and for mitochondrial mass of naïve CD8+ T cells versus VO_{2peak} ($r = 0.47$, $p = 0.009$) (1). A negative correlation was observed for naïve CD8+ T cell mitochondrial mass versus percent body fat ($r = -0.43$, $p = 0.017$) as measured by bioelectrical impedance analysis. Sex and body composition appear to be important factors in the previous relationships because correlations between T-cell mitochondrial mass and estimated energy expenditure or VO_{2peak} were no longer significant when controlling for percent body fat. Similarly, correlations between T-cell mitochondrial mass and estimated energy expenditure were no longer significant when controlling for sex (1). However, the positive relationship between CD8+ mitochondrial mass and VO_{2peak} remained significant ($r = 0.42$, $p = 0.024$).

Metabolic Function: In the previously mentioned report, Alley et al. also isolated naïve CD8+ T cells from PBMCs by immunomagnetic bead separation. They found no differences in glycolytic or mitochondrial function of anti-CD3/anti-CD28 co-stimulated naïve CD8+ T cells from participants in their high-fitness versus low-fitness groups, as measured by Seahorse assay (1).

Cell Function: Antunes et al. were the sole report to assess the effects of physical fitness on cell function of isolated leukocyte populations. In this report, monocytes from participants in their low-fitness group demonstrated greater LPS-stimulated (100 ng/mL) IL-10 production versus monocytes from those in the high-fitness group (3).

DISCUSSION

Overview

Research in the past two decades has demonstrated the integral role of cellular metabolism in directing immune cell phenotype and function. Furthermore, there are accumulating data from the field of exercise immunology revealing the ways exercise affects immune function via cellular metabolism. Given this context, the current review aimed to set a foundation for future work by summarizing the current body of literature regarding the effects of exercise and physical fitness on leukocyte energy metabolism. Figure 2 summarizes the number of studies that have been performed within different participant populations and by different experimental designs (e.g., acute exercise, physical fitness comparisons). The volume, breadth, and timespan of reports uncovered in the review were surprising

and suggest early and persistent interest in the metabolic effects of exercise on immune cells. However, while the diversity of reports provides an extensive resource to inspire additional research, it also presents some limitations to interpretation.

Limitations

The limitations of the literature are apparent when one attempts to reconcile the data against the heterogeneity of sample populations, types of exercise and/or training implemented, timepoints of blood collection in relation to exercise, and measurement techniques. We have attempted to resolve this heterogeneity by organizing the data based on exercise type and intensity, participant population, cell populations, and outcomes. The strength of this approach is that it efficiently reveals gaps in the literature; particularly regarding the effects of concurrent and resistance training. However, this organizational strategy in some cases may be incomplete, and all reports within a category may not provide appropriate comparisons. This strategy was chosen to find balance between organizational structure and dividing the dataset into sections too small for meaningful comparison. Given the objective of scoping reviews to be mainly descriptive (4, 56), the balance was weighted towards larger categories for the purpose of summary and comparison. However, we understand the diverse nature of the literature means our approach may not fit all aspects of the dataset equally and this remains a limitation of the review.

Additional limitations of the review include the possibility of missing reports and no weight of results relative to study quality. The first limitation is a consequence of methodological choices in the execution of the review. We attempted to cover a wide swath of the biomedical literature through our choice of databases. Nevertheless, it is possible relevant literature remain outside the reach of our search methods and therefore were not included in the review. This leaves the door open for future reviews to build on the current work. Also, though useful in weighing evidence between reports, critical analysis of study quality is not within the purview of scoping reviews and was therefore not implemented in this one. We encourage future systematic reviews and meta-analyses to implement such assessments to provide further clarity of research findings.

Acute Exercise Data

Despite the above-mentioned limitations of the current literature and this review, noteworthy trends and knowledge gaps were observable in the data. These results should offer direction for future research that we will now discuss.

Trends

Heavy-Severe Intensity Aerobic Exercise

Acute aerobic exercise of heavy-severe intensity was associated with changes in the expression of genes related to nutrient acquisition and mitochondrial respiration, including mitochondrial biogenesis. In general, increased gene expression was observed post-exercise. However, the timepoint of blood sampling appears important as positive results were more frequently observed in samples obtained >2h post-exercise. These trends were observed among athletes in mixed cell populations, and among healthy active participants in both mixed and isolated cell populations. In contrast, inconsistent results were observed regarding the effects of heavy-severe exercise on protein expression. This inconsistency may be attributable to the relatively fewer reports that assessed protein versus gene expression, or specifics of the participants and the exercise bout. Though the lone report to investigate the effects of acute heavy-severe aerobic exercise on metabolic regulation in inactive participants did not assess gene or protein expression, they did measure effects on mitochondria. Their observations that acute exercise increased the mitochondrial oxidant burden of NK cells, but did not alter mitochondrial count or MMP, implies that mitochondrial oxidative stress is a consequence of heavy-severe exercise among inactive participants (41).

Data obtained in athletes, healthy active, and inactive participants indicate acute heavy-severe intensity aerobic exercise alters peripheral leukocyte metabolic function. These changes were observed in both mixed cell populations, in monocytic cells incubated with post-exercise plasma in vitro, and in NK cells. However, it remains unclear whether observed changes in enzyme activity and substrate oxidation simply reflect shifts in substrate use versus change in total cellular metabolic activity. Furthermore, three reports suggest that exercise-induced changes in metabolic function may be due to exercise-induced changes in peripheral cell counts versus within-cell changes (44, 53, 64). These results were not observed in all reports, however, and it remains possible that within-cell changes may occur differentially between cell types. The report of Lin et al. provides some information to both of these questions, as increased ETS and Reserve OCR were observed post-exercise in NK cells (41). This implies NK cells are metabolically sensitive to acute exercise in sedentary men. Whether similar responses are observed in other cell types or participant populations remains to be determined.

Few reports investigated cell function in the context of acute heavy-severe aerobic exercise. Data from Busquets-Cortes et al. indicate increased stimulated intracellular ROS production in PBMCs alongside increased markers of mitochondrial regulation post-exercise (13). Further, Lin et al. report that exercise increased cytolytic mediators by NK cells alongside increased mitochondrial oxidative burden and increased ETS and Reserve OCR (42). However, the data are conflicted as to whether heavy-severe exercise has a stimulatory or inhibitory effect on measures of cell function, as lowered cell function is also reported (26, 49). Resolving this conflict will be important to determine whether exercise-induced changes in metabolic regulation and function, such as those described, imply promotion or suppression of immune function.

Light-Moderate Intensity Aerobic Exercise

Few reports investigated the effects of acute light-moderate aerobic exercise, and inactive participants with or without an ongoing disease were the only participant population studied. This perhaps is not surprising, given light-moderate exercise may be expected to deliver insufficient physiological stress to observe effects among healthy active participants or athletes. However, given the lack of data, whether this assumption is true remains unclear. Among inactive participants with or without disease, acute exercise was associated with depressed signaling for mitochondrial biogenesis, and mixed effects on mitochondrial regulation. However, unfortunately, methodological choices in the latter investigations direct that these results be interpreted with caution. Blood samples were acquired thirty minutes post-exercise in the report from Ferrer et al. (23), which the data on heavy-severe exercise suggests is too soon to observe

effects on PBMC gene expression. Additionally, though moderate, exercise in the reports from Tsai et al. and Chang and Wang was performed in hypoxic conditions (16, 66). Thus, the physiological stress of the bout was likely in excess of the moderate cycling workloads and therefore may not faithfully reflect the effects of moderate intensity cycling on PBMCs and lymphocytes.

Knowledge Gaps Regarding Acute Exercise

Major knowledge gaps regarding the effects of acute exercise on peripheral leukocyte metabolism and function include: effects of concurrent and resistance training, effects on glycolytic function, changes in substrate use versus overall metabolic rate, the influence of exercise intensity within individuals and between cell types. It is striking that no reports tested the effects of acute concurrent or resistance training bouts. These are major gaps in the literature that deserve attention due to the unique benefits each type of exercise offers. Additionally, few of the included reports assessed changes in the regulation or function of glycolysis. Given the dramatic changes in oxygen consumption and substrate utilization during acute exercise, and the role of glycolysis in facilitating immune cell activation, evaluating the regulation of glycolysis in leukocytes with exercise will be worthwhile. Related, evaluating activity of specific metabolic pathways in parallel with overall metabolic rate (e.g., oxygen consumption, ATP production) will be useful towards understanding if exercise only shifts leukocyte substrate use and/or alters metabolic rate. Such information will inform strategies (e.g., immunonutrition) to support immune function in the context of exercise or occupational physical activity. Finally, it is essential to resolve the effects of acute exercise on leukocyte metabolic function relative to exercise intensity and cell proportions. Contrasting the effects of variable exercise intensity within individuals, as well as between mixed versus isolated cell types, will help contextualize previous results and suggest if and how acute exercise can be used to modulate immunometabolism. These data will be instrumental to determining which cell types may be most metabolically sensitive to exercise and the immunometabolic effects imposed at different intensities.

Chronic Exercise Data

Trends

Heavy-Severe Intensity Aerobic Exercise

Heavy-severe aerobic exercise training was associated with changes in the expression of proteins regulating cellular metabolism in athletes and healthy active participants, but not sustained changes in gene expression. Among athletes, training led to increased expression of proteins that suggest signaling for mitochondrial biogenesis in mixed cell populations. Heavy-severe training in healthy active participants also led to increased protein expression, but for FA transport rather than mitochondrial biogenesis. The latter data stem from only one report, however, and therefore remain to be replicated for confirmation. In contrast to data obtained from athletes and healthy active participants, minimal changes in gene or protein expression of mixed cell populations were observed among inactive participants with or without disease following training. However, the change in ratio of mitochondrial dynamics proteins in one report imply increased mitochondrial fusion (66). In isolated cells, elevated MMP was noted post-HIIT training in two reports, alongside evidence for improved redox regulation (41). Taken together, the available data suggest heavy-severe aerobic exercise training signals for changes in mitochondrial homeostasis among mixed and isolated cells that may improve mitochondrial redox regulation. Though, training periods of six weeks or greater appear required to observe such changes.

The majority of data regarding the effects of heavy-severe aerobic exercise training on metabolic function were collected among inactive participants with or without disease, with too few results in athletes or healthy active participants for further commentary (Figure 2). In sum, data among inactive participants with or without disease indicate heavy-severe aerobic exercise training results in improvements to oxidative phosphorylation and respiratory capacity of peripheral leukocytes. Similar results were observed in both mixed cell population and isolated cell types; including T cells, neutrophils, and NK cells. However, data indicate that training must be in excess of two weeks to observe respiratory adaptations, as one report that only instituted a two-week training program observed no significant improvements to PBMC respiration (31). These trends are promising given the parallels to adaptations documented in skeletal muscle, and the importance of mitochondrial function to immune function (46).

Similar to the data on metabolic function, few reports assessed the effects heavy-severe aerobic exercise training on cell function. The two reports which investigated cell function following training in athletes observed minimal evidence of functional changes at rest in PBMCs, despite evidence of mitochondrial biogenesis signaling at the protein level (12, 13). In contrast, data obtained in inactive participants with or without disease indicate HIIT training can lead to improvements in cell function of both neutrophils and NK cells (6, 41). Notably, cell functional changes occurred alongside improvements to both cardiorespiratory fitness and cellular respiratory capacity, implying coordinated metabolic adaptation to exercise training.

Light-Moderate Intensity Aerobic Exercise

No reports investigated light-moderate training among athletes and only a single report on light-moderate aerobic exercise training in healthy active participants was recovered. The data of this latter report suggest light-moderate training is insufficient to alter cellular metabolism of lymphocytes, unless additional stimuli are provided (e.g., hypobaric hypoxia) (49). Though, these results deserve replication for confirmation given the limited data. In contrast, reports among inactive participants with or without disease were relatively more numerous. In mixed cells, greater expression of genes regulating lipid transport and oxidation were observed following training in two reports (14, 73), and decreased expression of proteins regulating mitochondrial dynamics were noted in one report (66). In isolated cells, training led to improvements in measures of mitochondrial function, both at rest and following acute heavy-severe intensity exercise (41). Together, the data indicate light-moderate training alters nutrient acquisition and mitochondrial homeostasis of peripheral leukocytes, with the potential for improved resilience to stresses such as exercise.

Data obtained in both healthy active and inactive participants with or without disease indicate light-moderate training can result in improvements in metabolic function of both mixed and isolated cell populations. Improvements in various markers of cell respiratory function were observed following training in lymphocytes of men diagnosed with occupational

burnout (10), in PBMCs of men with HIV (36), and in PBMCs, lymphocytes, or NK cells of sedentary but otherwise healthy young men (17, 41, 66). Two of these reports are notable for their investigations of acute exercise effects following training. Those data, obtained in one case in lymphocytes and in the other in isolated NK cells, indicate training-related changes mitigate the metabolic perturbations induced by acute exercise of peripheral lymphocytes (41, 66).

Only a single report was retrieved which evaluated the effects of light-moderate aerobic exercise training on cell function (41). These data were obtained in isolated NK cells and indicate light-moderate training may elicit equivalent changes in cell function as heavy-severe aerobic exercise training. Increased levels of granzyme b and perforin were recorded in NK cells of participants that completed light-moderate MICT or HIIT in this report, without differences between the groups. These results are promising and suggest light-moderate training may be sufficient stimulus to improve immune cell function among sedentary individuals. However, given this is but a single report, they also deserve follow up to determine the minimal as well as maximal effective intensities of aerobic exercise training in regard to peripheral leukocyte metabolism and function.

Knowledge Gaps Regarding Chronic Exercise

Despite revealing a number of trends worthy of comment, review of the data regarding the effects of exercise training on leukocyte metabolism and function also uncovered numerous gaps in knowledge. The most notable of these gaps include: the effects of concurrent and resistance training, the effects of training on different cell types obtained from the same participants, and the effects of exercise training on cellular glycolysis. Few reports investigated concurrent or resistance training at all, much less at different intensities, in different populations or cell types (Figure 2). Further, only a single study with healthy active participants employed a heavy-severe intensity training program, which might be expected to have a greater impact on cell metabolism and function. Although, this remains to be determined. The available data indicates these training styles are safe in both inactive and healthy participants, with promising results regarding metabolic regulation. Consequently, it will be important for future work to extend these studies to other populations (e.g., athletes), outcomes (metabolic function, cell function), and cell types to characterize the immunometabolic effects of resistance and the combination of aerobic and resistance exercise.

It also remains unclear how a given training program differentially affects various cells within the peripheral leukocyte pool. It is clear that metabolic differences exist between cell types and activation states (52, 59). However, whether differences also exist between cell types in regard to exercise and training remains unclear. Consequently, prospective comparisons of exercise training effects between different cell types will be extremely valuable for interpreting results coming from mixed populations and ascertaining whether certain cells are more or less "trainable." Finally, scant data were recovered concerning the effects of exercise training on leukocyte glycolytic function. As was stated previously when discussing acute exercise, glycolysis is integral to leukocyte activation and pathogen control, but is likewise linked to inflammation. Therefore, whether training improves or suppresses glycolytic function will have important implications toward the potential benefits and harms of pursuing exercise. In some cases, increasing cell activation and inflammatory potential may be helpful (e.g., recovering from sepsis), while in others it may be detrimental (e.g., chronic inflammatory disease). However, without knowledge of the effects of exercise training on leukocyte glycolysis it will be difficult to forecast its costs versus benefits.

Physical Fitness Data

Trends

Given the comparative methodological burden of acute exercise and training interventions, it was surprising that fewer cross-sectional reports on physical fitness were retrieved (Figure 2). Yet, the lack of reports leaves the door open for additional research and invites study of the relationships between physical fitness and leukocyte metabolism among many populations of interest. For example, no studies examined effects of physical fitness on metabolic regulation within populations with disease (Figure 2). With the caveat that there were relatively few data to draw upon, available data indicate higher fitness is associated with more stable mitochondrial function, greater substrate oxidation and respiratory enzyme activity among mixed cell populations. In parallel to these metabolic differences, cells of fitter individuals may also exhibit lower basal ROS production and proliferation, without compromised ROS and proliferation with maximal activation. Among isolated cell types, the data demonstrate positive associations between host fitness and signaling for lipid metabolism and mitochondrial biogenesis in monocytes (3), as well as mitochondrial mass in naïve T cells (1).

Knowledge Gaps Regarding Physical Fitness

A worthwhile avenue for future research will be defining the contexts in which differences in leukocyte metabolism present relative to physical fitness. Investigating individuals across the fitness continuum is an important aim to define if and how leukocyte metabolism scales with fitness and health. The thresholds of fitness for observing cellular differences may vary by health and activity status. Therefore, studies in diverse participant populations will provide context for interpreting the implications of leukocyte metabolism relative to fitness. It will also be valuable to document relationships between systemic and cellular metabolism of individuals of similar fitness, but differing in age or sex, and comparing cell types. Such data will inform how age- and sex-related effects change the latter relationships, and if the strength of relationships between systemic and cellular metabolism vary between leukocyte types. Finally, as was also true for acute exercise and chronic exercise, few studies of physical fitness assessed cell function alongside cell metabolism. Therefore, the impact of fitness-based differences in leukocyte metabolic regulation and function toward defense against pathogens and malignant cells remains opaque. Of all objectives, linking systemic and leukocyte metabolism with immune function may be the most important in regard to host health. We strongly recommend future investigations integrate measures of immune cell function (e.g., pro-/anti-inflammatory cytokine production, cytotoxicity) alongside metabolic assessments to situate cellular metabolic data relative to immune function and host health.

SUMMARY AND CONCLUSION

Effectively engaging cellular metabolic pathways is essential

to both exercise performance and immune function. Given the beneficial effects of exercise on systemic and skeletal muscle metabolic function, and on immunity, the potential for exercise to modulate immune function via leukocyte metabolism seems a logical possibility. The current literature suggest acute exercise can influence the regulation of leukocyte energy metabolism and metabolic function, and that the stimuli of training may enhance metabolic capacity (e.g., enhanced maximal oxidative phosphorylation capacity). However, there remain notable gaps in the literature to be filled with future research. These gaps include the effects of exercise and fitness on leukocyte glycolytic function, intensity and duration thresholds for exercise-induced leukocyte metabolic adaptations, as well as the existence and magnitude of differences in the metabolic response to exercise between cell types and subsets. In addition, relatively few studies examined cell function concurrently with measures of metabolic regulation or metabolic function. Ideally, future studies investigating acute and chronic exercise effects on cellular metabolism will include data on the regulation of cellular metabolism (e.g., protein expression, mitochondrial dynamics), metabolic function (e.g., mitochondrial respiration), as well as cell function (e.g., stimulated proliferation, cytokine production) to characterize the immediate and adaptive consequences of exercise and training. Such data will improve understanding of whether and how exercise affects immune function via cellular metabolism and inform the application of acute and chronic exercise to support both immune function and systemic health and well-being.

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Author, year	Table 2: Report Characteristics - Effects of Acute Exercise					
	Exercise Type and Intensity; Session	**Participants**	**Cell Population; Sampling Time Point**	**Participant Characteristics (N, % male, age)**	**Participant BMI, Body Composition**	**Fitness Assessment; Participant Fitness**
Capo X et al., 2020[b,c] (15)	Aerobic, heavy-severe intensity; 5 min. each @ running speed corresponding to 50%, 60%, 70% VO_{2max}, anaerobic threshold (to exhaustion)	Athletes (professional taekwondo)	PBMCs; Pre- vs post-exercise (2h)	11, 100%, N/R	N/R	VO_{2max} via treadmill GXT; mean ± SEM: 46.1 ± 4.8 ml/kg/min.
Ferry A, Marsac C, Duvallet A, Rieu M, 1991 (24)	Aerobic, heavy-severe intensity; Same as fitness test (i.e., treadmill GXT)	Athletes (endurance sports)	PBMCs; Pre- vs post-exercise	6, N/R, mean ± SD: 29.3 ± 2.0 y/o	N/R	VO_{2max} via treadmill GXT; mean ± SD: 68.7 ± 1.3 ml/kg/min.
Hunter DJ et al., 2019[b] (33)	Aerobic, heavy-severe intensity; 45 min. @ 70% W_{max} then 15 min. time-trial	Athletes (trained cyclists)	PBMCs; Pre- vs post-exercise	8, 100%, mean ± (error type unspecified): 39.50 ± 5.9 y/o	N/R	VO_{2max} via cycle ergometer GXT; mean ± (error type unspecified):53.88 ± 5.24 ml/kg/min.
Nieman DC et al., 2018[b] (51)	Aerobic, heavy-severe intensity; 75km indoor cycling time-trial	Athletes (trained cyclists)	Plasma; Pre- vs post-exercise (0 h, 0.75 h, 1.5 h, 3 h, 4.5 h, 21 h, 45 h post-exercise)	All: 20, 70%, N/R; mean ± SEM: F: 43.7 ± 2.2 y/o M: 37.1 ± 2.5 y/o	F: N/R, 18.8% BF M: N/R, 19.5% BF	VO_{2max} via cycle ergometer GXT; mean ± SEM: F: 46.5 ± 2.8 ml/kg/min., M: 47.0 ± 1.5 ml/kg/min.
Davies NA et al., 2015[b] (18)	Aerobic, heavy-severe intensity; 45 min. @ 70% VO_{2max}	Healthy, Active	PBMCs; Pre- vs post-exercise (0 h, 1.5 h, 3 h, 24 h post-exercise)	5, 100%, mean ± SD: 32 ± 8 y/o	N/R	VO_{2max} via cycle ergometer GXT; mean ± SD: 44 ± 14 ml/kg/min.
Frisina JP et al., 1994 (26)	Aerobic, heavy-severe intensity; 25 x 1 min. @ 112% maximal GXT pace, 2 min. rest	Healthy, Active	PBMCs; Pre- vs post-exercise	7, 100%, mean ± SD: 21.7 ± 1.3 6y/o	N/R	GXT to exhaustion via treadmill; N/R
Moir H et al., 2010 (48)	Aerobic, heavy-severe intensity; 45 min. cycling/running @ 70% VO_{2max}	Healthy, Active	PBMCs; Pre- vs post-exercise (0h, 1h)	Cycling: 16, 100%, mean ± SD: 23.1 ± 0.5 y/o Running: 8, 100%, 21.1 ± 1.2 y/o (subset of 16)	N/R	VO_{2max} via cycle ergometer GXT; mean ± SD: 56.3 ± 1.1 ml/kg/min.
Moir et al., 2008 (47)	Aerobic, heavy-severe intensity; 45 min. cycling @ 70% VO_{2max}	Healthy, Active	PBMCs; Pre- vs post-exercise (0 h, 1 h)	10, 100%, mean ± SD: 23.1 ± 0.5 y/o	N/R	VO_{2max} via cycle ergometer GXT; mean ± SD: 56.6 ± 1.1 ml/kg/min.
Theall B et al., 2021 (64)	Aerobic, heavy-severe intensity; 30 min. cycling @ watts corresponding to 65-75% VO_{2peak}	Healthy, activity N/R	PBMCs & T cells[d]; Pre- vs post-exercise	All: 21, 57%, mean ± SD: 27.0 ± 5.4 y/o F: 24.0 ± 3.9 y/o M: 29.3 ± 5.4 y/o (significantly different from F)	All: mean ± SD: 25.8 ± 4.0 kg/m^2; N/R F: 24.4 ± 2.9 kg/m^2; N/R M: 26.8 ± 4.5 kg/m^2; N/R (sig. different from F)	VO_{2peak} via cycle ergometer GXT; All: mean ± SD: 36.5 ± 6.3 ml/kg/min. F: 36.8 ± 3.1 ml/kg/min. M: 36.3 ± 8.1 ml/kg/min.
Meksawan K et al., 2005[b] (44)	Aerobic, heavy-severe intensity; Same as fitness assessment (i.e., treadmill GXT)	Healthy, Inactive	Leukocytes in whole blood; Pre- vs post-exercise	All: 10, 40%, N/R mean ± SEM: F: 22.3 ± 1.3 y/o M: 24.8 ± 1.0 y/o	mean ± SEM: F: 21.1 ± 0.6 kg/m^2, 25.9 ± 1.4% BF M: 23.7 ± 0.6 kg/m^2, 13.7 ± 1.4% BF	VO_{2peak} via treadmill GXT; mean ± SEM: F: 1.5 ± 0.1 L/min., M: 3.1 ± 0.1 L/min.
Chang SC & Wang JS, 2017[a] (16)	Aerobic, light-moderate intensity; 40 min. @ 60% VO_{2max} in hypoxia (12% O_2)	Healthy, Inactive	PBMCs; Pre- vs post-exercise	12, 100%, N/R	N/R	N/R
Ferrer MD et al., 2021[b] (23)	Aerobic, light-moderate intensity; 30 min. treadmill walk @ 60-70% HR_{max}	Overweight/obese men with MetS & BMI 27-40, capable of exercise	PBMCs; Pre- vs post-exercise (30 min.)	15, 100%, mean ± SEM: 66.5 ± 2.3 y/o	mean ± SEM: 29.3 ± 1.7 kg/m^2; WC: 102 ± 4 cm	N/R
Liepinsh E et al., 2020 (40)	Aerobic, light-moderate intensity; 60 min. exercise @ 50W	Healthy, Inactive	PBMCs; Pre- vs post-exercise	12, 42%, mean ± SEM: 36.2 ± 7.3 y/o	mean ± SEM: 25.1 ± 2.5 kg/m^2; N/R	Est. VO_{2max} via submaximal cycle ergometer GXT; mean ± SEM: 33.3 ± 1.3 ml/kg/min.
Bisset SK & Alexander WD, 1958 (7)	Aerobic, light-moderate intensity; 3-4 min. "moderate" treadmill exercise	Health and activity status N/R	PBMCs; Pre- vs post-exercise	6, N/R, 18-29 y/o	N/R	N/R

Notes: a= Data from conference abstract. b = data from placebo/control condition of study including dietary intervention. c = results relevant to this review taken from acute exercise session of larger study. d = T-cell nutrient transporter and metabolic enzyme expression analyzed from within PBMCs via Flow Cytometry. Abbreviations: %BF = body fat percentage; BMI = body mass index; BPM = beats per minute; F = female; GXT = graded exercise test; HR = heart rate; HR_{max}: maximum heart rate; M = male; MetS = metabolic syndrome; N/R = data relevant to review not reported; SD = standard deviation; SEM = standard error of the mean; VO_2 = volume of oxygen consumed per time unit; VO_{2max} = maximal volume of oxygen consumed per time unit; VO_{2peak} = peak volume of oxygen consumed per time unit; W_{max} = wattage at maximal aerobic exercise (VO_{2max}); WC = waist circumference; y/o = years old.

Author, year	Table 3: Report Characteristics - Effects of Exercise Training					
	Exercise Type and Intensity; Training	**Participants**	**Cell Population; Sampling Time Point**	**Participant Characteristics (N, % male, age)**	**Participant BMI, Body Composition**	**Fitness Assessment; Participant Fitness[d]**
Busquets-Cortes C et al., 2016[b] (12)	Aerobic, heavy-severe intensity; 8 weeks; 6 x 2 h soccer practice/week + 10 matches	Athletes (professional soccer players)	PBMCs; Pre- vs post- 8-week training	7, 100%, mean ± SEM: 18.9 ± 0.5 y/o	mean ± SEM: 23.1 ± 0.4 kg/m^2, mean ± SEM: 91.5 ± 0.3% FFM	N/R; mean ± SEM: 61.2 ± 1.6 ml/kg/min.
Gatterer H et al., 2018 (27)	Both groups: Aerobic, heavy-severe intensity; 3 weeks 3 days/week; cycle ergometer training in hypoxia RSH: 3 x 5 x10 s @ 85% BW w/ 20 s rest w/ 5 min. rest between series SIH: 4 x 30 s @ 0.75 x BW on 5 min. rest	Athletes (recreational)	PBMCs; Pre- vs post- 3-week training	All: 11, N/R, mean ± (error type not specified): 24.0 ± 2.4 y/o RSH: 6, N/R, 24.8 ± 2.5 y/o SIH: 5, N/R, 23.0 ± 2.1 y/o	N/R	*Wingate test:* RSH: mean ± (error type not specified): 811 ± 73 W, SIH: 789 ± 90 W *RS (5 x 6 s cycling sprints):* RSH: 1043 ± 89 W, SIH: 1000 ± 118 W *YYIR2:* RSH: 486.7 ± 134.9 m, SIH: 430.0 ± 50.3 m *RSA (6 x 34 m sprints w/ 20 s recovery):* RSH: 6.45 ± 0.36 s, SIH: 6.60 ± 0.26 s
Chang S & Wang J, 2015[a] (17)	Both groups: 6 weeks 5 days/week; 30 minutes cycling exercise: HIIT: Aerobic, heavy-severe intensity: alternating 3 min. @ 80% VO_{2max} & 40% VO_{2max} MICT: Aerobic, light-moderate intensity; continuous 60% VO_{2max}	Healthy, Inactive	Lymphocytes; Pre- vs post-training	HIIT: 12, 100%, N/R MICT: 12, 100%, N/R	N/R	N/R
Hasni S et al., 2021 (29)	Aerobic, heavy-severe intensity; 12 weeks 3 days/week; 30 min. treadmill exercise 70-80% $VO_{2reserve}$	SLE w/ fatigue, activity N/R	PBMCs; Pre- vs post-training	16, 0%, mean ± SD: 42.0 ± 10.3 y/o	N/R	Time to anaerobic threshold: N/R 10MWT; N/R
Hedges CP et al., 2019 (31)	Aerobic, heavy-severe intensity; 2 weeks, 6 training sessions; 8-12 x 60 seconds cycling @ W_{peak} interspersed with 75 seconds @ 30 watts	Healthy, Inactive	PBMCs; Pre- vs post-training	10, 100%, mean ± SEM: 24.7 ± 0.3 y/o	mean ± SEM: 24.1 ± 0.7 kg/m^2; N/R	VO_{2max} via cycle ergometer GXT; mean ± SEM: 3.3 ± 0.2 L/min.
Andonian B et al., 2020[a] (2)	Aerobic, heavy-severe intensity; 10 weeks high-intensity interval training	Rheumatoid arthritis, Inactive	T-cells; Pre- vs post-training	12 (n=6 PBMCs), N/R, N/R	N/R	N/R
Bartlett DB et al., 2020 (6)	Aerobic, heavy-severe intensity; 10 weeks 3 days/week; 20min. sessions alternating 60-90 seconds @ 80-90% $VO_{2reserve}$ vs. 60-90 seconds @ 50-60% $VO_{2reserve}$	Training group: Prediabetes, Inactive Control: Healthy, Active[c]	Neutrophils; Pre- vs post-training, vs control	Training group: 10, 40%, mean ± SD: 71 ± 5 y/o Control: 6, 50%, mean ± SD: 23 ± 1 y/o	Training group: mean ± SD: 29.4 ± 3.0 kg/m^2; 39.6 ± 8.6 % BF Control: mean ± SD: 25 ± 2.6 kg/m^2: N/R	Training group: VO_{2peak} via treadmill GXT; mean ± SD: 20 ± 2 ml/kg/min. 400 m walk test; 254 ± 27 s Berg balance scale; 54.3 ± 5.8 Grip strength; 27 ± 6.8 kg Timed-up and go; 8.9 ± 1.5 s

Morabito C et al., 2016 (49)	Aerobic, light-moderate intensity; 2 x12 days mountain trekking, ~6.5 h/day	Healthy, activity N/R	PBMCs; Pre- vs post-trekking at low altitude & high altitude	7, 0%, mean ± SEM: 36.3 ± 7.1 y/o	N/R	N/R
Brand S et al., 2020 (10)	Aerobic, light-moderate intensity; 12 weeks 3 days/week; supervised aerobic exercise @ 60-75% estimated HR_{max}	Training group: Self-reported occupational burnout, Inactive Control: Healthy, Inactive	Lymphocytes; Pre- vs post-training, vs control	Training group: 12, 100%, mean ± SD: 45.8 ± 6.8 y/o Control: 12, 100%, mean ± SD: 45.7 ± 5.7 y/o	N/R	N/R
Butcher LR et al., 2008 (14)	Aerobic, light-moderate intensity; 8 weeks 3 days/week; 10,000 steps on treadmill @ self-selected pace	Healthy, Inactive	PBMCs; Pre- vs post-training	All: 34, 53%, mean ± SD: 45.6 ± 11.1y/o Training: 17, N/R, mean ± SD: 44.94 ± 10.1 y/o Control: 17, N/R, mean ± SD: 46.12 ± 12.2 y/o	Training: mean ± SD: 26.78 ± 5.11 kg/m^2, 33.78 ± 10.25% BF Control: mean ± SD: 27.02 ± 5.32 kg/m^2, 34.15 ± 10.03% BF	Estimated VO_{2max} via Rockport submaximal treadmill test; Training: mean ± SD: 35.49 ± 6.49 ml/kg/min. Control: mean ± SD: 34.62 ± 5.68 ml/kg/min.
Kocher M et al., 2017 (36)	Aerobic, light-moderate intensity; 12 weeks; 20 min./session @ 50-80% HR_{max} increased 2 min./week to 40 min./session	HIV^+, Inactive	PBMCs; Pre- vs post- training	7, 100%, 36-58 y/o	Median ± (error type not specified): 24.6 ± 4.2 kg/m^2	VO_{2peak} via cycle ergometer GXT; N/R
Yakeu G et al., 2010 (73)	Aerobic, light-moderate intensity; 8 weeks 3 days/week; 10,000 steps on treadmill at self-selected pace	Healthy, Inactive	PBMCs; Pre- vs mid- vs post-training	17, 53%, mean ± SD: 45.6 ± 11.1 y/o	mean ± SD: 26.78 ± 5.11 kg/m^2	N/R
Lehti M et al., 2020 (38)	Concurrent Exercise; 21 weeks 2 days/week; MIAST @ 50-60% $HR_{reserve}$ (later increased to 85%) for 1h	Coronary artery disease, Inactive	PBMCs; Pre- vs post-training, training vs control	Training: 12, 92%, mean ± SD: 58.6 ± 8.5 y/o Control: 11, 82%, mean ± SD: 63.3 ± 6.1 y/o	Training: mean ± SD: 27.1 ± 3.0 kg/m^2 Control: mean ± SD: 27.9 ± 4.2 kg/m^2	VO_{2peak} via cycle ergometer; Training: mean ± SD: 25.8 ± 5.3 ml/kg/min. Control: mean ± SD: 23.2 ± 4.9 ml/kg/min.
Estebanez B et al., 2019 (21)	Resistance Exercise; 8 weeks 2 days/week; full body RT 3 x 12/8/12 repetitions of 8 exercises	Heathy, Active (resistance training naïve)	PBMCs; RT experiment: Pre- vs post- 8-week RT (old), Aging: baseline old vs young	RT experiment: All: 30, 37%, mean ± SEM: 72.8 ± 2.2 y/o Training: 20, N/R, mean ± SEM: 73.7 ± 2.2 y/o Control: 10, N/R, mean ± SEM: 73.8 ± 2.3 y/o Young: 10, N/R, mean ± SEM: 22.5 ± 2.3 y/o	RT experiment: Training: mean ± SEM: 27.5 ± 0.7 kg/m^2 Control: mean ± SEM: 28.5 ± 0.8 kg/m^2 Young: mean ± SEM: 24.6 ± 2.5 kg/m^2	MVIC for leg press & bicep curl; Training: mean ± SEM: 76.0 ± 5 kg, 48.5 ± 4.3 kg Control: N/R 1RM for leg press, bicep curl, pec deck; Training: mean ± SEM: 71.0 ± 4.7 kg, N/R, 55.0 ± 6.9 kg Control: N/R

Notes: a = Data from conference abstract; b = data from placebo/control condition of study including dietary intervention, or in which all participants received same dietary treatment; c = healthy highly active participants only used for baseline comparisons; d = baseline fitness data. Abbreviations: 1RM = 1-repetition maximum; 10MWT = 10-meter walk test; %BF = body fat percentage; BMI = body mass index; BW = body weight; %FFM = fat-free mass percentage; GXT = graded exercise test; HIIT = high-intensity interval training; HR_{max} = maximal heart rate; $HR_{reserve}$ = heart rate reserve, HR_{max} - HR_{rest}; MICT = moderate-intensity continuous training; MIAST = mixed interval-type aerobic and strength training; MVIC = maximal voluntary isometric contraction; N/R = data relevant to review not reported; PBMC = peripheral blood mononuclear cell; RSA = repeated sprint ability (running); RSH = repeated-sprint training in hypoxia; RT = resistance training; RS = repeated cycling sprints; SD = standard deviation; SEM = standard error of the mean; SIH = sprint-interval training in hypoxia; SLE = systemic lupus erythematosus; VO_{2max} = maximal volume of oxygen consumed per time unit; VO_{2peak} = peak volume of oxygen consumed per time unit; $VO_{2reserve}$ = reserve VO_2, VO_{2max} -VO_{2rest}; W_{peak} = peak wattage during exercise test; y/o = years old; YYIR2 = yo-yo intermittent recovery test level 2

Author, year	Table 4: Report Characteristics - Effects of Physical Fitness				
	Participants	**Cell Population; Comparator(s)**	**Participant Characteristics (N, % male, age)**	**Participant BMI, Body Composition**	**Fitness Assessment; Participant Fitness**
Alley JR, Valentine RJ, Kohut ML, 2022 (1)	Healthy, Inactive and Active	RBC-lysed blood, PBMCs; PA level/ CRF	Inactive: 15, 53%, mean ± SD: 24 ± 6 y/o Active: 15, 47%, mean ± SD: 23 ± 3 y/o	Inactive: mean ± SD: 21.7 ± 1.5 kg/m^2, 23.6 ± 5.3% BF Active: mean ± SD: 21.6 ± 1.5 kg/m^2, 15.6 ± 4.0% BF	VO_{2peak} via treadmill GXT; Inactive: mean ± SD: 42.7 ± 7.4 ml/kg/min. Active: mean ± SD: 59.6 ± 9.0 ml/kg/min.
Antunes BM et al., 2020[a] (3)	Healthy, Inactive and Active	Monocytes; CRF	22, 100%, mean ± (error type not specified):25.8 ± 5.7 y/o	N/R	VO_{2max} (ml/kg/min.) via cycle ergometer GXT; Low-fit: mean: 35.3 ml/kg/min. High-fit: mean: 63.1 ml/kg/min.
Dorneles GP et al., 2021[a] (20)	Healthy, Inactive and Active	PBMCs; CRF	22, 100%, mean ± SD: 26.8 ± 3.1 y/o	Low-fit: mean ± SD: 23.9 ± 1.2 kg/m^2, 81.2 ± 7.8 cm WC Moderately-fit: mean ± SD: 23.5 ± 1.1 kg/m^2, 80.7 ± 4.1cm WC High-fit: mean ± SD: 23.8 ± 1.6 kg/m^2, 78.1 ± 6.3 cm WC	VO_{2peak} via treadmill GXT; Low-fit: mean ± SD: 38.1 ± 1.7 ml/kg/min. Moderately-fit: mean ± SD: 43.9 ± 2.7 ml/kg/min. High-fit: mean ± SD: 55.1 ± 4.7 ml/kg/min.
Mota MP et al., 2010 (50)	Healthy, Inactive and Active	Lymphocytes; CRF, age	Low fit young (LFY): 8, 100%, mean ± SD: 20.8 ± 1.0 y/o High fit young (HFY): 13, 100%, mean ± SD: 23.0 ± 3.7 y/o Low fit adult (LFA): 9, 100%, mean ± SD: 37.0 ± 3.6 y/o High fit adult (HFA): 9, 100%, mean ± SD: 32.8 ± 3.4 y/o Low fit middle aged (LFM): 13, 100%, mean ± SD: 48.9 ± 5.4 y/o High fit middle aged (HFM): 14, 100%, mean ± SD: 46.1 ± 3.5 y/o	N/R	VO_{2max} via treadmill GXT; (all mean ± SD): LFY: 42.9 ± 5.1 ml/kg/min. HFY: 57.4 ± 3.9 ml/kg/min. LFA: 38.5 ± 1.9 ml/kg/min. HFA: 51.3 ± 3.3 ml/kg/min. LFM: 33.9 ± 5.5 ml/kg/min. HFM: 49.1 ± 5.5 ml/kg/min.
Vladutiu GD et al., 2002 (69)	4 sisters w/ genetic CPT2 deficiency; Healthy Inactive; Healthy Athlete	Leukocytes in whole blood; Health status, training status	Sisters: 4, 0%, 24-30 Inactive: 20, 0%, mean ± (error type not specified): 24 ± 3 y/o Athlete: 24, 0%, mean ± (error type not specified): 33 ± 2 y/o	N/R	VO_{2max} via treadmill GXT; Sisters: range: 36.5-43.6 ml/kg/min. Inactive: 34.3 ml/kg/min. (central tendency not specified) Athlete: 47.9 ml/kg/min. (central tendency not specified)
Farinha JB et al., 2015 (22)	Overweight or obese, Inactive and Active	PBMCs; PA level	All: 35, 0%, N/R (Postmenopausal) Inactive: 12, 0%, mean ± SD: 56.25 ± 4.76 y/o Active: 23, 0%, mean ± SD: 53.82 ± 5.49 y/o	All: BMI >25 kg/m^2 Inactive: mean ± SD: 32.99 ± 4.38 kg/m^2, 97.58 ± 11.13 cm WC Active: mean ± SD: 31.07 ± 5.12 kg/m^2, 90.29 ± 10.68 cm WC	VO_{2max} via treadmill GXT; Inactive: mean ± SD: 18.6 ± 2.01 ml/kg/min. Active: mean ± SD: 22.22 ± 4.67 ml/kg/min.
Tyrrell DJ et al., 2015 (67)	Overweight or obese, Inactive	PBMCs; Physical fitness	15, 60%, mean ± SD: 68.3 ± 3.5 y/o	mean ± SD: 30.8 ± 2.4 kg/m^2; 8.3 ± 1.6 kg leg lean mass	Ex-SPPB; mean ± SD: 2.5 ± 0.3 400 m walk test; mean ± SD: 1.5 ± 0.5 m/s Grip strength; mean ± SD: 35.6 ± 10.7 kg Knee extension; mean ± SD: 122.0 ± 36.0 Nm

a=results relevant to this review taken from a single phase of larger study. Abbreviations: %BF = body fat percentage; BMI = body mass index; CPT2 = carnitine palmitoyltransferase 2; CRF=cardiorespiratory fitness; Ex-SPPB = expanded short physical performance battery score; GXT= graded exercise test; HFA = high-fit adult group; HFM = high-fit middle-aged adult group; HFY = high-fit young adult group; LFA = low-fit adult group; LFM = low-fit middle-aged adult group; LFY = low-fit young adult group; N/R = data relevant to review not reported; PA = physical activity; RBC= red blood cell; SD = standard deviation; VO_2 = volume of oxygen consumed per time unit; VO_{2max} = peak volume of oxygen consumed per time unit; VO_{2peak} = peak volume of oxygen consumed per time unit; WC = waist circumference; y/o = years old.

Author, year	Table 5: Report Characteristics - Effects of Acute Exercise plus Physical Fitness or Exercise Training						
	Exercise Type and Intensity; Session	**Exercise Type and Intensity; Training**	**Participants**	**Cell Population; Sampling Time Points; Comparators**	**Participant Characteristics (N, % male, age)**	**Participant BMI, Body Composition**	**Fitness Assessmenta nd Participant Fitness[a]**
Janssen JJE et al., 2022 (34)	Aerobic, heavy-severe intensity; 60 min. cycle ergometer @ 70% VO_{2peak}	N/A	Healthy, Low-fit & High-fit	PBMCs; CRF, Pre- vs post-exercise (21h)	Low-fit: 16, 0%, median [IQR]: 24.0 y/o [21.3-25.5] High-fit: 15, 0%, median [IQR]: 21.8 y/o [21.6-23.7]	Low-fit: N/R, mean ± SD: 28.9 ± 3.9% BF High-fit: N/R, mean ± SD: 25.1 ± 4.4% BF	VO_{2max} via cycle ergometer GXT; Low-fit: median [IQR]: 35.1 ml/kg/min. [32.2-35.7] High-fit: median [IQR]: 50.4 ml/kg/min. [49.0-54.0]
Pendergast DR et al., 2004 (53)	Aerobic, heavy-severe intensity; GXT to exhaustion via treadmill	N/A	Four groups: Afflicted with disease; Healthy, inactive; Healthy, athletes (elite runners, recreational runners)	Leukocytes in whole blood; Health/ training status, pre- vs post-exercise	Inactive: 43, N/R, 20-40 y/o Inactive + Acute Exercise: 12, 42%, 20-30 y/o Active: 12, 50%, 27-45 y/o Athletes: 5, 100%, 26-32 y/o CPT2 def: 2, 0%, 24 & 29 y/o MS: 31, 16%, 30-45 y/o CFS: 6, 0%, 30-45 y/o OB: 5, 40%, 18-35 y/o ED: 16, 0%, 16-25 y/o	N/R	N/R
Busquets-Cortes C et al., 2017 (13)	Aerobic, heavy-severe intensity; Leger-Boucher test pre- and post-training + sport drills post-training	Aerobic, heavy-severe intensity; 8 weeks, 5 x 2 h soccer practice + 1 game/week	Athletes (professional soccer players)	PBMCs; Pre- vs post-training, pre- vs post-exercise (2 h)	12, 100%, mean ± SEM: 19.3 ± 0.4 y/o	mean ± SEM: 24.0 ± 0.6 kg/m^2, mean ± SEM: 92.5 ± 0.2% FFM	Estimated VO_{2max} via Leger-Boucher test; mean ± SEM: 60.4 ± 1.8 ml/kg/min.
Thomas AW et al., 2012 (65)	Aerobic, heavy-severe intensity; 45 min. cycle ergometer @ 70% VO_{2max}	Aerobic, heavy-severe intensity; 8 weeks 2-4 sessions/weekc ycle ergometer @ 60-85% VO_{2max} for 30-60 min.	Healthy, Active	PBMCs; Pre- vs post-training, pre- vs post-exercise	Acute exercise: 9, N/R, mean ± SEM: 32 ± 8 y/o Training: 8, N/R, mean ± SEM:27.8 ± 6.4 y/o	N/R	VO_{2max} via cycle ergometer GXT pre - & post-training; N/R

Lin ML et al., 2022 (41)	Aerobic, heavy-severe intensity; same as exercise test (GXT)	Both: 6 weeks 5 days/week cycle training: HIIT: Aerobic, heavy-severe intensity: 5 x 3 min. @ 80% VO_{2max} then 3 min. @ 40% VO_{2max}; or MICT: Aerobic light-moderate intensity: 30 min. @ 60% VO_{2max}	Healthy, Inactive	NK cells; Pre- vs post-training, training protocol	HIIT: 20, 100%, mean ± SEM: 22.2 ± 2.1 y/o MICT: 20, 100%, mean ± SEM: 22.3 ± 2.8 y/o Control: 20, 100%, mean ± SEM: 22.6 ± 2.7 y/o	mean ± SEM: HIIT: 24.3 ± 3.1 kg/m^2, N/R MICT: 23.3 ± 2.9 kg/m^2, N/R Control: 24.0 ± 2.8 kg/m^2, N/R	VO_{2max} via cycle ergometer GXT pre- & post-training; mean ± SEM: HIIT: 33.5 ± 4.8 ml/kg/min. MICT: 33.2 ± 4.5 ml/kg/min. Control: 32.2 ± 4.1 ml/kg/min.
Tsai HH et al., 2016 (66)	Aerobic, light-moderate intensity; 36 min. cycling @ 50-100w in hypoxia (12% O_2)	Both: 6 weeks 5 days/week cycle training: HIIT: Aerobic, heavy-severe intensity: 5 x 3 min. @ 80% VO_{2max} then 3 min. @ 40% VO_{2max} MICT: Aerobic light-moderate intensity 30min. @ 60% VO_{2max}	Healthy, Inactive	Lymphocytes; Pre- vs post-training, pre- vs post-hypoxic exercise, training protocol	HIIT: 20, 100%, mean ± SEM: 22.2 ± 2.1 y/o MICT: 20, 100%, mean ± SEM: 22.3 ± 2.8 y/o Control: 20, 100%, mean ± SEM: 22.6 ± 2.7 y/o	mean ± SEM: HIIT: 22.5 ± 0.6 kg/m^2; N/R MICT: 22.6 ± 0.7 kg/m^2; N/R Control: 22.7 ± 0.6 kg/m^2; N/R	VO_{2max} via cycle ergometer GXT pre- & post-training; mean ± SEM: HIIT: 34.0 ± 1.4 ml/kg/min. MICT: 33.1 ± 1.2 ml/kg/min. Control: 32.2 ± 1.0 ml/kg/min.

Notes: a= baseline fitness data. Abbreviations: %BF = body fat percentage; BMI = body mass index; CFS = individuals with chronic fatigue syndrome; CPT2 def.= individuals with carnitine palmitoyltransferase 2 deficiency; CRF = cardiorespiratory fitness; ED = individuals with eating disorder, anorexia nervosa; %FFM = fat-free mass percentage; GXT = graded exercise test; HIIT = high-intensity interval training; IQR = interquartile range; MICT = moderate intensity continuous training; MS = individuals with multiple sclerosis; N/A = not applicable; N/R = not reported; OB = individuals with obesity; SD = standard deviation; SEM = standard error of the mean; VO_{2max} = maximal volume of oxygen consumed per time unit; VO_{2peak} = peak volume of oxygen consumed per time unit; y/o = years old.

Author, Year	Table 6: Key Findings - Reports Evaluating Effects of Acute Exercise			
	Cell Number and Phenotype	**Metabolic Regulation**	**Metabolic Function**	**Cell Function**
Heavy-Severe Intensity AE				
Busquets-Cortes C et al., 2017[c] (13)	No difference in # PBMC's or % lymphocytes, % monocytes (2 h post-exercise)	↑PBMC COXIV, PGC-1α, MitND5 mRNA expression (2 h post-exercise) ↑PBMC UCP2, PGC-1α, Mfn-2 protein expression (2 h post-exercise)	N/R	↑PBMC intracellular ROS and H_2O_2 production in PMA-stimulated PBMCs (2 h post-exercise) ↑NF-κB activation, (2 h post-exercise)
Capo X et al., 2020[b,c] (15)	N/R	↑PBMC SIRT3 mRNA expression (2 h post-exercise)	N/R	N/R
Ferry A, Marsac C, Duvallet A, Rieu M, 1991 (24)	N/R	N/R	Mean ± SD: ↓33.6 ± 6.6% lymphocyte PDHc activity ↑43.1 ± 4.7% lymphocyte CS activity No change lymphocyte COX or SCR activity per mg protein	N/R
Hunter DJ et al., 2019[b] (33)	N/R	↓ PBMC PGC-1α gene methylation ↑PBMC PGC-1α mRNA expression +Correlation PBMC PGC-1α gene methylation vs. TT cycling mean power ($\rho = 0.714$, $p < 0.05$) -Correlation PBMC PGC-1α gene methylation vs. PBMC protein carbonyls ($\rho = -0.714$, $p < 0.05$)	N/R	N/R
Nieman DC et al., 2018[b] (Water-only vs. CHO-conditions) (51)	#Leukocytes > CHO-conditions immediately, 0.75 h, 1.5 h, 3 h, 4.5 h post-exercise	N/R	↓THP-1 average OCR following water-only plasma incubation (6 h) vs. CHO-conditions (immediately post-exercise plasma) THP-1 SRC following water-only plasma incubation (37.9%) < CHO-conditions (avg. 95.8%) (immediately post-exercise plasma) ↑THP-1 average ECAR following water-only plasma incubation (6 h) vs. CHO-conditions (immediately post-exercise plasma)	No change THP-1 COX-2 expression following culture (6 h) with immediately post-, 1.5h post-, or 21h post-exercise plasma vs. pre-exercise Greater THP-1 COX-2 expression with water-only 21 h plasma vs. CHO food conditions.
Janssen JJE et al., 2022[c] (34)	↓# PBMCs (21 h post-exercise) ↓% $CD4^+CD25^+$ T cells and $CD4^+CD25^+$ $CD127^+$ T cells (low-fit only) (21 h post-exercise)	N/R	No change in PBMC OCR or PER (21 h post-exercise)	N/R
Pendergast DR et al., 2004[c] (53)	↑#leukocytes in sedentary & elite runners	N/R	↑Total leukocytes FA oxidation in sedentary No difference leukocytes FA oxidation per cell in sedentary No change total leukocytes FA oxidation in elite runners ↓leukocytes FA oxidation per cell in elite runners	N/R
Davies NA et al., 2015[b] (18)	N/R	↑PBMC PGC-1α, PPARγ, ABCA1 mRNA expression (3 h post-exercise) No change PBMC AMPK protein phosphorylation (3 h post-exercise)	N/R	N/R

Frisina JP et al., 1994 (26)	↓$CD3^+$ & $CD20^+$ cells, $CD4^+$:$CD8^+$ ratio (3 min. post-exercise) ↑$CD56^+$ cells (3 min. post-exercise)	N/R	↑18.69% lymphocyte glutamine oxidation (3 min. post-exercise) ↑27.02% lymphocyte lactate production (3 min. post-exercise) +Correlation glutamine oxidation vs. %change NK cells ($r = 0.78$, $p < 0.01$) -Correlation glutamine oxidation vs. %change T cells ($r = -0.93$, $p < 0.001$) -Correlation lactate production vs. %change T cells ($r = -0.66$, $p < 0.01$)	↓58% lymphocyte Con-A (3.5 μg/mL, 48hrs) stimulated proliferation (3 min. post-exercise) +Correlation %change proliferation vs. %change T cells ($r = 0.78$, $p < 0.01$) -Correlation %change proliferation vs. %change NK cells ($r = -0.76$, $p < 0.05$)
Moir H et al., 2010 (48)	↑#leukocytes 0 h, 1 h	↓PBMC PGC-1α mRNA expression (0 h post-exercise) No change PBMC PGC-1α mRNA expression (1 h post-exercise) ↓PBMC AMPK protein phosphorylation (0 h post-exercise) No change PBMC AMPK protein phosphorylation (1 h post-exercise)	N/R	↓PBMC Unstimulated and PMA-stimulated (25 ng/mL, 10 min) ROS (0 h post-exercise)
Moir et al., 2008 (47)	↑#leukocytes 0 h, 1 h	No change Monocyte CD36 mRNA expression (0 h, 1 h post-exercise) ↓Monocyte AMPK protein phosphorylation (0 h post-exercise) No change Monocyte AMPK protein phosphorylation (1 h post-exercise) No change intracellular ATP	N/R	N/R
Thomas AW et al., 2012[c] (65)	N/R	↑PBMC CD36 & ABCA1 mRNA expression (3 h post-exercise)	N/R	N/R
Theall B et al., 2022 (64)	↑%, #naïve ($KLRG^-$/$CD57^-$) and senescent ($KLRG^+$/$CD57^+$) $CD8^+$ T cells ↑#very-early ($CD69^+$), early ($CD25^+$), and late ($CD71^+$) activated $CD8^+$ T cells ↑#early ($CD25^+$) and late ($CD71^+$) activated $CD4^+$ T cells ↑% but not # $CD38^+$ (late activation) $CD4^+$ and $CD8^+$ T cells	↑#$GLUT4^+$ $CD4^+$ T cells ↓%$CD36^+$ CD8, % $HK2^+$ $CD4^+$, % and # $HK1^+$ $CD4^+$, % $HK1^+$ $CD8^+$ T cells +Correlation # $CD69^+$ and $CD25^+$ $CD8^+$ T cells vs. # $GLUT1^+$ $CD8^+$ T cells ($\rho = 0.672$, p 0.001 & $\rho = 0.391$, $p = 0.088$) +Correlation $CD69^+$ and $CD38^+$ $CD4^+$ T cells vs. $GLUT1^+$ $CD4^+$ T cells ($\rho = 0.528$ & $\rho = 0.577$) +Correlation # $CD38^+$ $CD4^+$ T cells vs. $HK1^+$ $CD4^+$ T cells ($\rho = 0.556$, $p = 0.039$) +Correlation $CD71^+$ and $CD38^+$ $CD4^+$ T cells vs. $CD36^+$ $CD4^+$ T cells ($\rho = 0.635$, $p = 0.003$ & $\rho = 0.630$, $p = 0.003$)	No change PBMC routine, leak, OXPHOS, ETS & RCRs per 10^6 cells ↑Routine ($d = 0.58$), routine (PMG) ($d = 0.77$), Leak (PMGS) ($d = 0.80$), OXPHOS ($d = 0.80$), ETS ($d = 0.78$) respiration per mL blood	N/R
Meksawan K et al., 2005[b] (44)	↑#leukocytes	N/R	↓leukocyte FA oxidation per cell ↑leukocyte FA oxidation per mL blood	N/R
Lin ML et al., 2022[c] (41)	↑#NK cell ↑#$CD45RA^+$ & $CD57^+$ NK cell ↓#$CD45RO^+$ NK cell	No change NK cell mitochondrial content or MMP ↑NK cell MOB	↑NK cell ETS & Reserve OCR & BHI	↑NK cell Perforin & Granzyme B

Light-Moderate Intensity AE				
Chang SC & Wang JS, 2017[a] (16)	N/R	↑PBMC MMP	No change PBMC routine or maximal OXPHOS respiration ↓PBMC CI respiration	N/R
Ferrer MD et al., 2021[b] (23)	N/R	↓PBMC COXIV & Tfam mRNA expression (30 min. post-exercise) No change PBMC UCP3, MnSOD, COXIV, Mtf1, Mtf2 protein expression (30 min. post-exercise)	N/R	N/R
Liepinsh E et al., 2020[b] (40)	N/R	N/R	↑31% PBMC routine respiration (15 min. post-exercise) ↑65% PBMC FA-dependent leak respiration (15 min. post-exercise) ↑76% PBMC FA-dependent OXPHOS (15 min. post-exercise) ↑22% PBMC FA coupling efficiency (15 min. post-exercise)	N/R
Tsai HH et al., 2016[c] (66)	↑#Lymphocyte & %$CD57^+$ lymphocytes ↓% $CD62L^+$ & $CD28^+$ lymphocytes	No change mitochondrial biogenesis or metabolic regulatory proteins No change in mitofusin or Drp-1 No change lymphocyte mitochondrial count ↓lymphocyte MMP, ↑MOB	↓Lymphocyte ATP-linked OCR, reserve OCR, OCR via CI and CII substrates ↑Lymphocyte LDH & GDH activity ↓ Lymphocyte CS activity	N/R
Bisset SK & Alexander WD, 1958 (7)	No change #leukocytes (5 min. post-exercise)	N/R	↑36-71% leukocyte VO_2 (5 min. post-exercise)	N/R

Notes: Data derived from blood samples obtained immediately post-exercise unless otherwise noted. a = Data from conference abstract. b = Data from placebo/control condition of study including dietary intervention, or in which all participants received same treatment. c = acute exercise data from larger study. Abbreviations: ABCA1 = ATP binding cassette subfamily A member 1; AMPK = AMP-activated protein kinase; ATP = adenosine triphosphate; BHI = bioenergetic health index; CHO = carbohydrate; CI = complex 1 of mitochondrial electron transport chain; CII = complex II of mitochondrial electron transport chain; Con-A = concanavalin A; COX = cytochrome c oxidase; COXIV = cytochrome C oxidase subunit 4; CS = citrate synthase; Drp-1 = dynamin-related protein 1; ETS = electron transport system; FA = fatty acid; GDH = glutamate dehydrogenase; LDH = lactate dehydrogenase; Mfn-2 = mitofusin-2; MitND5 = mitochondrial NADH dehydrogenase subunit 5; MMP = mitochondrial membrane potential; MOB = mitochondrial oxidant burden; NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells; N/R = data relevant to review not reported; OCR = oxygen consumption rate; OXPHOS = oxidative phosphorylation; PBMC = peripheral blood mononuclear cells; PER = proton efflux rate; PDHc = pyruvate dehydrogenase complex; PGC-1α = peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARγ = peroxisome proliferator-activated receptor gamma; RCR = respiratory control ratio; ROS = reactive oxygen species; SCR = succinate cytochrome reductase; SD = standard deviation; SIRT3 = NAD-dependent deacetylase sirtuin-3, mitochondrial; SRC = spare respiratory capacity; Tfam = transcription factor A, mitochondrial; THP-1 = human monocytic cell line; TT = time trial; UCP2 = uncoupling protein 2; VO_2 = volume of oxygen consumed per time.

Author, Year	Table 7: Key Findings-Reports Evaluating Effects of Exercise Training				
	Training Response	**Cell Number, Proportion, and Phenotype**	**Metabolic Regulation**	**Metabolic Function**	**Cell Function**
Heavy-Severe Intensity AE training					
Busquets-Cortes C et al., 2016[b] (12)	N/R	No change # PBMCs, % lymphocytes, % monocytes	↑PBMC Tfam, OPA1, OMA1 protein expression	N/R	No change PMA-stimulated (10 ng/mL) PBMC H_2O_2 production ↓PBMC MDA ↑PBMC protein carbonyls
Busquets-Cortes C et al., 2017[g] (13)	No change estimated VO_{2max} pre vs. post 8 wks training	No change in # PBMC's or % lymphocytes, % monocytes	No change PBMC COXIV, PGC-1α, MitND5 gene expression ↑PBMC UCP2, UCP3, COXIV, Mfn-1, and ↓PBMC Tfam protein expression	N/R	No change PBMC NF-κB activation, PMA-stimulated (10 ng/mL) H_2O_2 production
Gatterer H et al., 2018 (27)	↑YYIR2 running distance, RSA performance (RSH & SIH) ↑Wingate & RS mean and peak power (RSH & SIH) ↑RS skeletal muscle deoxygenation and re-oxygenation(RSH)	N/R	N/R	↓PBMC ETS per cell (RSH, n=2) ↓ETS normalized to CS activity (RSH & SIH, n=2/group)	N/R
Thomas AW et al., 2012[g] (65)	↓HR, blood lactate during submaximal exercise test No change VO_{2max}	N/R	↑PBMC CD36 protein expression No change PBMC PPARγ, phosphor-PPARγ:non-phospho-PPARγ protein expression	N/R	N/R
Hasni S et al. 2021[a] (29)	↑Time to anaerobic threshold, 10MWT performance	N/R	N/R	↑PBMC OCR/ECAR -Correlation FSS scores vs. OCR/ECAR	N/R
Hedges CP et al., 2018 (31)	↑VO_{2peak} (L/min)	No change #leukocytes, lymphocytes, #monocytes	No change PBMC PGC-1α, Tfam, NRF1, NRF2, COXIV gene expression No change PBMC mitochondrial CI-CV protein expression	No change PBMC mitochondrial respiration No correlation ΔPBMC mitochondrial respiration vs. Δskeletal muscle mitochondrial respiration	N/R
Andonian B et al., 2020[a] (2)	N/R	N/R	N/R	+Correlation ΔCRF vs. $\Delta CD4^+$ T cell basal and maximal respiration (ρ = 0.89, both) +Correlation $\Delta CD4^+$ T cell mitochondrial respiration vs. $\Delta CD4^+CCR7^+CD45RA^+$ T cells (ρ = 0.89)	N/R
Bartlett et al., 2020 (6)	Training group: ↑16 ± 11% VO_{2peak} ↓400m walk time	Training group: ↓#leukocytes (p = 0.059) No change # neutrophils	Training group: ↑Neutrophil MMP (d = 1.10)	Training group: ↑Neutrophil basal respiration (d = 3.01), maximal respiration (d = 2.86), ATP production (d = 4.18) ↓Neutrophil proton leak (n = 5) (d = 2.02)	Training group: ↑Neutrophil E. coli phagocytosis (d = 0.85), PMA-stimulated (25nM) ROS (d = 0.98), chemotaxis (d = 0.97), chemotactic index (d = 1.12)

					↓Neutrophil basal ROS (d = 0.93) +Correlation %ΔVO_{2peak} vs. neutrophil chemotaxis (r = 0.649, p = 0.042) -Correlation Δbody fat vs. neutrophil chemotaxis (r = -0.721, p = 0.018)
Heavy-Severe Intensity AE and Light-Moderate Intensity AE training Groups					
Chang SC & Wang JS, 2015[a] (17)	N/R	N/R	N/R	↑Lymphocyte ATP-linked OCR & ↓non-mitochondrial OCR (HIIT & MICT) ↑Lymphocyte pyruvate + glutamate-mediated OCR (HIIT only) ↑Lymphocyte succinate- & palmitoyl carnitine-mediated OCR (MICT only)	N/R
Lin ML et al., 2022[g] (41)	↑Vt, $Watts_{max}$, VE_{max}, VO_{2max}, VCO_{2max} (HIIT & MICT) HIIT Δ$Watts_{max}$, ΔVE_{max} > MICT	HIIT & MICT: ↑Resting & post-exercise #total & CD56dim NK cells ↓Resting & post-exercise %$CD45RA^+$ & $CD57^+$ NK cells ↑Resting & Post-Exercise #$CD45RO^+$ NK cells	HIIT & MICT: ↑Mitochondrial content & MMP @ rest & post-exercise ↓MOB post-exercise	↑NK cell ETS, Reserve OCR, BHI @ rest (HIIT & MICT) +Correlation ΔVO_{2max} vs. ΔNK cell ETS (r = 0.549) & Reserve OCR (r = 0.655) & BHI (r = 0.546)	↑NK cell perforin and granzyme b @ rest (HIIT & MICT)
Tsai HH et al., 2016[g] (66)	↑Watts, HR, VE, VO_2, VCO_2 @ V_t, exercise time to V_t (HIIT & MICT) ↑$Watts_{max}$, VE_{max}, $VO_{2max,}$ VCO_{2max}, time to max (HIIT & MICT) HIIT ΔCardio-pulmonary measures, Δtime to V_t, Δtime to max > MICT	HIIT & MICT: ↓HE-induced rise in total lymphocytes ↑% $CD28^+$, ↓%$CD57^+$ lymphocytes @ rest No change%$CD11a^+$, $CD45RA^+$, or $CD54RO^+$ cells @ rest or post-HE HIIT only: ↑%$CD28^+$,↓%$CD57^+$ lymphocytes post-HE	HIIT & MICT: No change mitochondrial biogenesis proteins ↓mitofusin & Drp-1 ↓HE-induced change MMP ↓HE-induced MOB HIIT only: ↑mitofusin: Drp-1 @ rest and immediately post-exercise ↑MMP @ rest & post-HE	HIIT & MICT: ↑ATP-linked OCR and Reserve OCR @ rest and post-HE ↑CII-linked OCR and OXPHOS capacity @ rest, ↓HE-induced respiratory changes ↓HE-induced changes in LDH & CS activity ↑SDH activity @ rest & post-HE	N/R
Light-Moderate Intensity AE training					
Morabito C et al., 2016 (49)	N/R	low-ALT: No change # PBMCs ↑% $CD69^+$ $CD4^+$ and $CD8^+$ T cells high-ALT: ↑% B cells, % $CD25^-CD69^-$ B cells, ↓% T cells, ↑% $CD69^+$ $CD8^+$ T cells	low-, high-ALT: No change PBMC HIF-1α protein expression No change MMP high-ALT: ↓mtROS among total & $CD3^+$	high-ALT: ↑PBMC metabolic activity via MTT staining	low-, high-ALT: No change intracellular ROS or NO
Brand S et al., 2020 (10)	N/R	N/R	N/R	Training group: ↑Lymphocyte mitochondrial OXPHOS (d = 1.27) & ETS (d = 1.67) respiration, Complex IV activity (d = 1.08) at Post-training Training group: Lymphocyte ATP content < Control at Baseline (d = 1.4),	N/R

				increased pre- to post-training (d = 0.91) -Correlation Lymphocyte ATP content vs. burnout score across sample (r = -0.62, p < 0.001)	
Butcher LR et al., 2008 (14)	Training: No change VO_{2max} (mean ± SD: +2.74 ± 4.21 ml/kg/min) Control: No change VO_{2max} (mean ± SD: +1.53 ± 2.63 ml/kg/min)	N/R	Training: PBMC CD36 & PPARγ gene expression @ 4 wks, 8 wks > Control or Baseline Training: PBMC ABCA1, ABCG1 gene expression @ 8 wks > Control or Baseline	N/R	N/R
Kocher M et al., 2017 (36)	↑14% VO_{2max}	No change in #$CD4^+$ T cells, viral load	N/R	↑PBMC non-mitochondrial respiration, respiratory capacity, SRC	N/R
Yakeu G et al., 2010 (73)	N/R	N/R	↑PBMC PGC-1α, PPARα gene expression @ 4 & 8 wks ↑PBMC PGC-1β gene expression @ 8 wks	N/R	N/R
Other Exercise Training					
Lehti M et al., 2020 (38)	Training group ↑VO_{2peak} at 12 weeks, trend (p = 0.06) for VO_{2peak} time x group effect (training vs. control) Training group ↑knee extensor MVIC 0 vs. 21 & 12 vs. 21 wks, time x group effect (p = 0.02)	N/R	Training group: ↑PBMC OXPHOS gene expression (z=3.27) (n=7)	N/R	N/R
Estebanez B et al., 2019 (21)	Training group ↑leg press, bicep curl MVIC + 1RM	N/R	Training group ↑PBMC PGC-1α, Mfn1 protein expression	N/R	N/R

Notes: a = Data from conference abstract; b = data from placebo/control condition of study including dietary intervention, or in which all participants received same dietary treatment; g = training results of a larger study. Abbreviations: 10MWT = 10-meter walk test; 1RM = 1-repitition maximum; ABCA1 = ATP binding cassette subfamily A member 1; ABCG1 = ATP binding cassette subfamily G member 1; ATP = adenosine triphosphate; BHI = bioenergetic health index; CI-CV = complexes 1-5 of the mitochondrial electron transport chain; CII = complex 2 of the mitochondrial electron transport chain; COXIV = cytochrome C oxidase subunit 4; CRF = cardiorespiratory fitness; CS = citrate synthase; Drp-1 = dynamin-related protein 1; ECAR = extracellular acidification rate; ETS = electron transport system; Ex-SPPB = expanded short physical performance battery; FSS = fatigue severity scale; HE = hypoxic exercise, exercise in 12% O_2; high-ALT = high-altitude trekking; HIF-1α = hypoxia-inducible factor-one subunit alpha; HIIT = high-intensity interval training; HR = heart rate; LDH = lactate dehydrogenase; low-ALT = low-altitude trekking; MDA = malondialdehyde; Mfn = mitofusin-1; MICT = moderate-intensity continuous training; MitND5 = mitochondrial NADH dehydrogenase subunit 5; MMP = mitochondrial membrane potential; MOB = mitochondrial oxidant burden; mtROS = mitochondrial reactive oxygen species; MTT = 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; MVIC = maximal voluntary isometric contraction; N/R = data relevant to review not reported; NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells; NO = nitric oxide; NRF1 = nuclear respiratory factor 1; NRF2 = nuclear respiratory factor 2; OCR = oxygen consumption rate; OMA1 = Metalloendopeptidase OMA1, mitochondrial; OPA1 = OPA1 mitochondrial dynamin like GTPase; OXPHOS = oxidative phosphorylation; PBMC = peripheral blood mononuclear cells; PGC-1α = peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PGC-1β = peroxisome proliferator-activated receptor beta; PPARα = peroxisome proliferator-activated receptor alpha; PPARγ = peroxisome proliferator-activated receptor gamma; ROS = reactive oxygen species; RS = repeated cycling sprints; RSA = repeated sprint ability (running); RSH = repeated-sprint training in hypoxia; RT = resistance training; SD = standard deviation; SDH = succinate dehydrogenase; SIH = sprint-interval training in hypoxia; SRC = spare respiratory capacity; tAT = time to anaerobic threshold; Tfam = transcription factor A, mitochondrial; UCP2 = uncoupling protein 2; UCP3 = uncoupling protein 3; VE = minute ventilation; VE_{max} = minute ventilation at maximal exercise; VCO_2 = volume of carbon dioxide expired per unit time; VCO_{2max} = maximal volume of carbon dioxide expired per unit time; VO_2 = volume of oxygen consumed per time unit; V_t = ventilatory threshold; $Watts_{max}$ = cycling power at maximal exercise; YYIR2 = yo-yo intermittent recovery test level 2.

Author(s), Year	Table 8: Key Findings - Reports Evaluating Effects of Physical Fitness			
	Cell Number, Proportion, and Phenotype	**Metabolic Regulation**	**Metabolic Function**	**Cell Function**
Reports examining apparently healthy individuals				
Alley JR, Valentine RJ, Kohut ML, 2022 (1)	No difference # naïve $CD4^+$ & $CD8^+$ T cells b/w groups	(N=7/group) Active naïve $CD8^+$ T-cell mitochondrial mass > Inactive (d = 0.76) Naïve $CD4^+$ & $CD8^+$ T-cell MMP, and $CD8^+$ T-cell mitochondrial biogenesis not different b/w groups +Correlation Naïve $CD4^+$ & $CD8^+$ T-cell mitochondrial mass vs. EE (r = 0.41, p = 0.024 & r = 0.36, p = 0.048); effect abrogated when controlled for sex or %BF +Correlation Naïve $CD8^+$ T-cell mitochondrial mass vs. VO_{2peak} (r = 0.47, p = 0.009); effect abrogated when controlled for %BF -Correlation Naïve $CD8^+$ T-cell mitochondrial mass vs. %BF (r = -0.43, p = 0.017)	(N=7/group) ECAR and mitochondrial OCR not different between groups	N/R
Antunes BM et al., 2020[a] (3)	No difference %monocyte subsets Low-fit vs. High-fit	High-fit LPS-induced (100 ng/mL) monocyte PPARγ expression > Low-fit High-fit monocyte PGC-1α expression > Low-fit all treatments (η^2 = 0.513) Low-fit monocyte AMPK expression > High-fit all treatments (η^2 = 0.372)	N/R	Low-fit LPS-induced (100 ng/mL) monocyte IL-10 > High-fit (p = 0.08)
Dorneles GP et al., 2021[a] (20)	N/R	High-fit & Moderately-fit Con-A (10 μg/mL) stimulated mitochondrial membrane depolarization < Low-fit	N/R	Low-fit unstimulated and Con-A (5 ug/mL) stimulated ROS production > Moderately-fit & High-fit High-fit unstimulated and Con-A (5 ug/mL) stimulated proliferation < Moderately-fit & Low-fit
Janssen JJE et al., 2022[b] (34)	No difference # PBMC b/w groups High-fit $\%CD14^+$ monocytes, $CD4^+CD25^+$ & $CD4^+CD25^+CD127^+$ T cells > Low-fit	N/R	High-fit basal OCR, Max OCR, SRC, ATP-linked OCR, proton leak > Low-fit High-fit Con-A (25 μg/mL) stimulated basal & maximal OCR > Low-fit No group difference in Unstimulated, Pre- vs. Post-Exercise, or Con-A (25 μg/mL) stimulated PER	N/R
Mota MP et al, 2010 (50)	N/R	HF lymphocyte mitochondrial H_2O_2 < LF (Y, A, M)	LFY lymphocyte CI activity > HF (Y,A) but no difference LFM vs. HFM -Correlation LF lymphocyte CI activity vs. age (r = -0.47, p-value N/R) LFY lymphocyte CI activity > LFM No difference HFY vs. HFM lymphocyte CI activity	N/R
Reports examining apparently healthy individuals and those with disease				

Pendergast DR et al., 2004[g] (53)	N/R	N/R	No difference leukocyte FA oxidation Inactive vs. Non-elite Runners Elite Runner resting leukocyte FA oxidation > Inactive & Non-Elite Runners CPT2 def. leukocyte FA oxidation < all other groups MS, ED, OB leukocyte FA oxidation = Sedentary CFS leukocyte FA oxidation > Sedentary	N/R
Vladutiu GD et al., 2002 (69)	N/R	N/R	CPT2-def. leukocyte FA oxidation < Sedentary, Trained Trained leukocyte FA oxidation > Sedentary +Correlation leukocyte FA oxidation vs. VO_2 @ RER = 1.0 (r = 0.94, p-value N/R)	N/R
Reports examining individuals with overweight or obesity				
Farinha JB et al., 2015 (22)	N/R	N/R	No difference Active vs Inactive in PBMC CI & CII activity, PBMC non-mitochondrial metabolic activity	Active PBMC ROS, SOD activity, and CAT activity > Inactive
Tyrell DJ et al., 2015 (67)	N/R	N/R	+Correlation PBMC SRC & maximal OCR vs. Ex-SPPB score (r = 0.59, r = 0.58), grip strength (r = 0.54, r = 0.52), knee extensor strength (r = 0.60, r = 0.60), leg skeletal muscle quality (SRC only) (r = 0.56) +Correlation PBMC basal respiration vs. knee extensor strength (r = 0.51) -Correlation PBMC SRC, maximal respiration, basal respiration vs. plasma IL-6 (r = -0.55, r = -0.58, r = -0.61)	N/R

Notes: a = results relevant to this review taken from a single phase of larger study. b = fitness effect data from larger study. Abbreviations: A = Adult group; AMPK = AMP-activated protein kinase; ATP = adenosine triphosphate; %BF = body fat percentage; b/w = between; CI = complex 1 of the mitochondrial electron transport chain; CII = complex 2 of the mitochondrial electron transport chain; CAT = catalase; CFS = individuals with chronic fatigue syndrome; Con-A = concanavalin A; CPT2 def.= individuals with genetic carnitine palmitoyltransferase 2 deficiency; ECAR = extracellular acidification rate; ED = individuals with eating disorder, anorexia nervosa; EE = energy expenditure; Ex-SPPB = expanded short physical performance battery; FA = fatty acid; HF = high-fitness group; HFA = high-fit adult group; HFM = high-fit middle-aged adult group; HFY = high-fit young adult group; IL-"X" = interleukin-"X"; LF = low-fitness group; LFA = low-fit adult group; LFM = low-fit middle-aged adult group; LFY = low-fit young adult group; LPS = lipopolysaccharide; M = Middle-aged adult group; MMP = mitochondrial membrane potential; MS = individuals with Multiple Sclerosis; N/R = data relevant to review not reported; OB = individuals with obesity; OCR = oxygen consumption rate; OXPHOS = oxidative phosphorylation; PBMC = peripheral blood mononuclear cells; PER = proton efflux rate; PGC-1α = peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARγ = peroxisome proliferator-activated receptor gamma; RER = respiratory exchange ratio; ROS = reactive oxygen species; SOD = superoxide dismutase; SRC = spare respiratory capacity; TNF = tumor necrosis factor; VO_2 = volume of oxygen consumed per time unit; VO_{2peak} = peak volume of oxygen consumed per time unit; Y = Young adult group.

Supplementary Table 1 – Review of Potentially Overlapping Review Articles			
Title	Subject	Overlap	Not Overlap
Immunmetabolism-fit: How exercise and training can modify T cell and macrophage metabolism in health and disease. (PMID: 35452394)	Review of phenotypic and metabolic features of T cells and macrophages, as well as what is known regarding their response to exercise	Clear topic overlap	Narrative review, not systematic Limited to T cells and macrophages
Influence of Obesity and Weight Loss on the Bioregulation of Innate/Inflammatory Responses: Macrophages and Immunometabolism (DOI: 10.3390/nu14030612)	Review of inflammatory dysregulation in obesity, concept of bioregulation of innate immunity (i.e., regulation of inflammatory potential via exercise), speculation regarding whether nutritional interventions can offer similar effects via weight loss	Regulatory role of exercise on immune function, with emphasis on metabolism	Limited to innate immunity Narrative review
Costly immunometabolic remodelling in disused muscle buildup through physical exercise (DOI: 10.1111/apha.13782)	Review of skeletal muscle and systemic consequences of atrophy and potential for exercise to rectify maladaptive changes	Use of exercise to remediate maladaptive immunometabolic changes with inactivity	Narrative review focused on the problem of muscle atrophy due to inadequate loading, and potential solutions to this problem
Type and Intensity as Key Variable of Exercise in Metainflammation Diseases: A Review (DOI: 10.1055/a-1720-0369)	Review of the effects of exercise and training on monocytes and lymphocytes (T-cells) in people with Obesity/MetS/ Diabetes	Effects of acute and chronic exercise on monocyte and lymphocyte bioenergetics	Narrative review focused on people with obesity/MetS/ Diabetes Limited focus on cellular metabolism
Does Modern Lifestyle Favor Neuroimmunometabolic Changes? A Path to Obesity (DOI: 10.3389/fnut.2021.705545)	Discussion of the impact of sedentary lifestyle and poor diet on systemic inflammation, and how this translates to neuroinflammation	Relationship between lifestyle, energy substrate use, inflammation	Perspective piece, anchored in the role of neuro-inflammation on path to obesity
Exercise and the Adrenergic Regulation of Immunity (DOI: 10.1016/j.bbi.2021.07.010)	Effects of exercise and training on mobilization of leukocytes and the role of adrenergic signaling in this process. Potential for exercise to modulate adrenergic signaling and improve responses to stress.	Effects of catecholamines on immunometabolism could yield some overlap	Narrative review focused on adrenergic signaling rather than exercise immuno-metabolism
Immunometabolic responses according to physical fitness status and lifelong exercise during aging: New roads for exercise immunology (DOI: 10.1016/j.arr.2021.101341)	Summary of adverse immunometabolic consequences of aging and inactivity, verified and potential benefit effects of exercise	Immunometabolism in aging and obesity, influence of exercise on these relationships	Narrative review with emphasis on the effects of aging specifically Effects of acute versus chronic exercise not well segregated
Mitochondrial Functionality in Inflammatory Pathology – Modulatory Role of Physical Activity (DOI: 10.3390/life11010061)	Review of maladaptive changes to systemic inflammation and OS with aging and metabolic disease, discussion of exercise effects on macrophages via mitochondrial signaling, implications towards mitigating systemic inflammation and compromised immune function	Effects of exercise on circulating monocytes and resident macrophages	Narrative review focused on mitochondrial signaling within macrophages
Endurance Exercise Mitigates Immunometabolic Adipose Tissue Disturbances in Cancer and Obesity (DOI: 10.3390/ijms21249745)	Review of the adverse adipose tissue changes in obesity and effects of exercise on modulating tissue inflammation in obesity and cancer	Effects of exercise on leukocyte metabolism and phenotype in adipose tissue	Focus on the effects of exercise on adipose tissue in the context of obesity and cancer Emphsis on cytokine and cell subset effects
SARS-CoV-2 and mitochondrial health: implications of lifestyle and aging (DOI: 10.1186/s12979-020-00204-x)	Influence of immune cell mitochondrial function on overall immune function, necessity of hormesis to maintain mitochondrial function	Lifestyle effects on mitochondrial health, implications to immune function	Not review of exercise effects on immune cell metabolism
Muscle-Organ Crosstalk: Focus on Immunometabolism (DOI: 10.3389/fphys.2020.567881)	Review of muscle-organ signaling, as modified by exercise and training. Short discussion of role(s) for myokines in immunometabolism.	Muscle-derived signaling molecules (myokines) that mediate effects of exercise throughout the body	Review of interorgan signaling via myokines, not focused on the effects of exercise on immune cell metabolism

Impact of Exercise on Immunometabolism in Multiple Sclerosis (DOI: 10.3390/jcm9093038)	Review of adverse immunometabolic changes in MS, potential for exercise to induce an anti-inflammatory state, and future research directions for the use of exercise in MS patients	Immunometabolic changes due to exercise that lead to an anti-inflammatory bias	Strictly limited to context of MS
Exercise of immunometabolic regulation in Cancer (DOI: 10.1038/s42255-020-00277-4)	Effects of exercise on organ systems that participate in carcinogenesis and on the TME.	Changes in cell phenotype and cytokine production associated with exercise	Exercise effects on metabolism and exercise effects on immune system discussed separately
Exercise immunology: Future directions (DOI: 10.1016/j.jshs.2019.12.003)	Summary of data from field of exercise immunology to date, potential utility of immunmetabolic outcome measures with examples from recent data	Discussion of both the effects of exercise on immunity and the role of cellular metabolism in directing immune cell form and function.	Cellular metabolism relates current state of the science and future directions for research rather than literature review
The Exercise Training Modulatory Effects on the Obesity-Induced Immunometabolic Dysfunctions (DOI: 10.2147/DMSO.S234992)	Review of adverse metabolic and immune changes in obesity with emphasis on monocyte recruitment and macrophage polarization, and how exercise may ameliorate or exacerbate meta-inflammation	Exercise effects on cytokine gene expression patterns and the number and phenotype of circulating cells	Emphasis on the pathogenesis and pathological consequences of obesity-induced chronic inflammation
New Insights about Regulatory T Cells Distribution and Function with Exercise: The Role of Immunometabolism (DOI: 10.2174/1381612826666200305125210)	Review of impact of exercise on circulating regulatory T cell numbers and functions, with implications towards combatting systemic inflammation. Particular consideration is given to how metabolic changes associated with exercise training may be affecting regulatory T cell function.	Metabolic stimulus of exercise and its potential effects on regulatory T cell number and function Role of cellular metabolism in regulatory T cell function	Limited to discussion of regulatory T cells
Nutrients, immune system, ad exercise: Where will it take us? (DOI: 10.1016/j.nut.2018.09.019)	Discussion of innate and adaptive immune-cell metabolism and how cellular metabolism can be affected by exercise and nutrients	Effect of exercise on metabolic signaling in immune cells plus its impacts on cell differentiation	Narrative review
Immunometabolism: A Multi-Omics Approach to Interpreting the Influence of Exercise and Diet on the Immune System (DOI: 10.1146/annurev-food-032818-121316)	Presentation of immunometaboilsm as a framework for integrating multiple analytical techniques to assess the acute and chronic effects of exercise on the immune system.	Effects of exercise on immune cell number and functional measures	Heavy emphasis on metabolomics and immuno-nutrition, less emphasis on cellular metabolism
Macrophage Polarization: Implications on Metabolic Diseases and the Role of Exercise (DOI: 10.1615/CritRevEukaryotGeneExpr.2016015920)	Review of the role of macrophages in metabolic diseases and inflammation, effects of exercise on macrophage polarization	Effect of exercise on macrophage gene expression and metabolism via cell polarization	Limited to macrophages Emphasis on metabolic disease

Supplementary Table 2: List of Potentially Relevant References Excluded from Analysis with Reason for Exclusion				
Article title	Journal	Authors	Year	Reason for exclusion
The Effects of Mitochondrial DNA Deletion and Copy Number Variations on Different Exercise Intensities in Highly Trained Swimmers	Cellular and Molecular Biology	O. Baykara, S.K. Sahin, F. Akbas, M. Guven, I. Onaran	2016	Does not meet review independent variable criteria (participant not adults)
Peripheral Blood Mononuclear Cells Antioxidant Adaptations to Regular Physical Activity in Elderly People	Nutrients	C. Busquets-Cortés et al.	2018	Does not meet review independent variable criteria (no exercise or physical fitness component)
Molecular choreography of acute exercise	Cell	K. Contrepois et al.	2020	Does not meet review dependent variable criteria (no leukocyte energy metabolism data)
Antioxidant Regulatory Mechanisms in Neutrophils and Lymphocytes After Intense Exercise	Journal of Sports Sciences	M.D. Ferrer, P. Tauler, A. Sureda, J.A. Tur, A. Pons	2009	Does not meet review independent variable criteria (participants not adults)
Alpha-Lipoic Acid Supplementation Increases the Efficacy of Exercise- and Diet-Induced Obesity Treatment and Induces Immunometabolic Changes in Female Mice and Women	The Federation of American Societies for Experimental Biology Journal	S. Le Garf et al.	2020	Does not meet review dependent variable criteria (insufficient data regarding effect of exercise specifically)
Effects of 6-Week Specific Low-Intensity training on Selected Aerobic Capacity Parameters and HSPA1A, HSPB1, and LDHb Gene Expression in High-Level Rowers	Genetics and Molecular Research	Z. Jastrzebski, M. Zychowska	2015	Missing data
The effect of mitochondrial energetics inhibitors on spontaneous rosette formation of lymphocytes from athletes	Journal of Sports Medicine and Physical Fitness	J.I. Karpova, E.N. Mokhova, N.I. Volkov	1987	Does not meet review dependent variable criteria (no leukocyte energy metabolism data)
Cycling Exercise Training Enhances Platelet Mitochondria Bioenergetics in Patients with Peripheral Arterial Disease: A Randomized Controlled Trial	Thrombosis and Haemostasis	M.L. Lin et al.	2021	Does not meet review dependent variable criteria (no leukocyte energy metabolism data)
Immune adaptation to chronic intense exercise training: new microarray evidence	BMC Genomics	D. Liu et al.	2017	Does not meet review dependent variable criteria (data from whole blood, not leukocytes)
No Effect of Resveratrol in Patients with Mitochondrial Myopathy: A Cross-over Randomized Controlled Trial	Journal of Inherited Metabolic Disease	N. Lokken et al.	2021	Does not meet review dependent variable criteria (insufficient data for unique exercise effect)
Affected pathways and transcriptional regulators in gene expression response to an ultra-marathon trail: Global and independent activity approaches	PLOS One	M. Maqueda, E. Roca, D. Brotons, J.M. Soria, A. Perera	2017	Does not meet review dependent variable criteria (data from whole blood, not leukocytes)
Sixteen-Week Physical Activity Intervention in Subjects With Increased Cardiometabolic Risk Shifts Innate Immune Function Towards a Less Proinflammatory State	Journal of the American Heart Association	M.P. Noz et al.	2019	Does not meet review independent variable criteria (physical activity, not exercise data)
Moderate to vigorous physical activity volume is an important factor for managing nonalcoholic fatty liver disease: A retrospective study	Hepatology	S. Oh et al.	2015	Does not meet review independent variable criteria (physical activity, not exercise data)

Influence of Cardiorespiratory Fitness on PPARG mRNA Expression Using Monozygotic Twin Case Control	Journal of Diabetes Research	M.R. Queiroga et al.	2015	Does not meet review independent variable criteria (participant adults)
Genomic signatures of a global fitness index in a multi-ethnic cohort of women	Annals of human genetics	E. Rampersaud et al.	2013	Does not meet review dependent variable criteria (data from whole blood, not leukocytes)
Mitochondrial reactive oxygen species generation in blood cells is associated with disease severity and exercise intolerance in heart failure patients	Nature- Scientific Reports	R. Shirakawa et al.	2019	Does not meet review independent variable criteria (comparisons by disease status, not exercise or physical fitness)
Patients with chronic fatigue syndrome performed worse than controls in a controlled repeated exercise study despite a normal oxidative phosphorylation capacity	Journal of Translational Medicine	R.C.W. Vermeulen, R.M. Kurk, F.C. Visser, W. Sluiter, H.R. Scholte	2010	Does not meet review independent variable criteria (comparisons by disease status, not exercise or physical fitness)
High-Intensity Interval Training Improves Mitochondrial Function and Suppresses Thrombin Generation in Platelets Undergoing Hypoxic Stress	Nature- Scientific Reports	L.-H. Wu, S.-C. Chang, T.-C. Fu, C.-H. Huang, J.-S. Wang	2017	Does not meet review dependent variable criteria (no leukocyte energy metabolism data)
Do Blood Cells Mimic Gene Expression Profile Alterations Known to Occur in Muscular Adaptation to Endurance Training?	European Journal of Applied Physiology	J. Zeibig, H. Karlic, A. Lohninger, R. Dumsgaard, G. Smekal	2005	Does not meet review independent variable criteria (participant adults)

Combined exercise intervention in a mouse model of high-risk neuroblastoma: effects on physical, immune, tumor and clinical outcomes.

Cecilia Rincón-Castanedo[1], Asunción Martín-Ruiz[1,2*], Sandra Zazo[3*], Ana L. Luis Huertas[4], Pedro L. Valenzuela[5,6], María Morán[7,8], Steven J. Fleck[1], Alejandro Santos-Lozano[5,9], Manuel Ramírez[10,11,12], Federico Rojo[3], Alejandro Lucia[1,5], África González-Murillo[10,11,12**], Carmen Fiuza-Luces[5**]**

[*]These two authors share the same position
[**]Share senior authorship

[1] Faculty of Sport Sciences, Universidad Europea de Madrid, Madrid, Spain
[2] Biophysics & Systems Biology Group. Department of Biochemistry and Molecular Biology, Complutense University of Madrid (UCM). Madrid, Spain
[3] Pathology, Fundación Jiménez Díaz University, Hospital Health Research Institute (IIS—FJD, UAM)—CIBERONC, Madrid, Spain
[4] Servicio de Cirugía Pediátrica, Hospital Infantil Universitario Niño Jesús, Madrid, Spain
[5] Physical Activity and Health Research Group ('PAHERG'), Instituto de Investigación Sanitaria Hospital '12 de Octubre' ('imas12'), Madrid, Spain
[6] Department of Systems Biology, University of Alcalá, Alcalá de Henares, Spain
[7] Mitochondrial and Neuromuscular Diseases Laboratory, Instituto de Investigación Sanitaria Hospital '12 de Octubre' ('imas12'), Madrid, Spain
[8] Spanish Network for Biomedical Research in Rare Diseases (CIBERER), U723, Madrid, Spain
[9] i+HeALTH, Department of Health Sciences, European University Miguel de Cervantes, Valladolid, Spain
[10] Unidad de Terapias Avanzadas, Oncología, Hospital Infantil Universitario Niño Jesús, Madrid, Spain
[11] Fundación de Investigación Biomédica, Hospital Infantil Universitario Niño Jesús, Madrid, Spain
[12] Instituto de Investigación Sanitaria La Princesa, Madrid, Spain

ABSTRACT

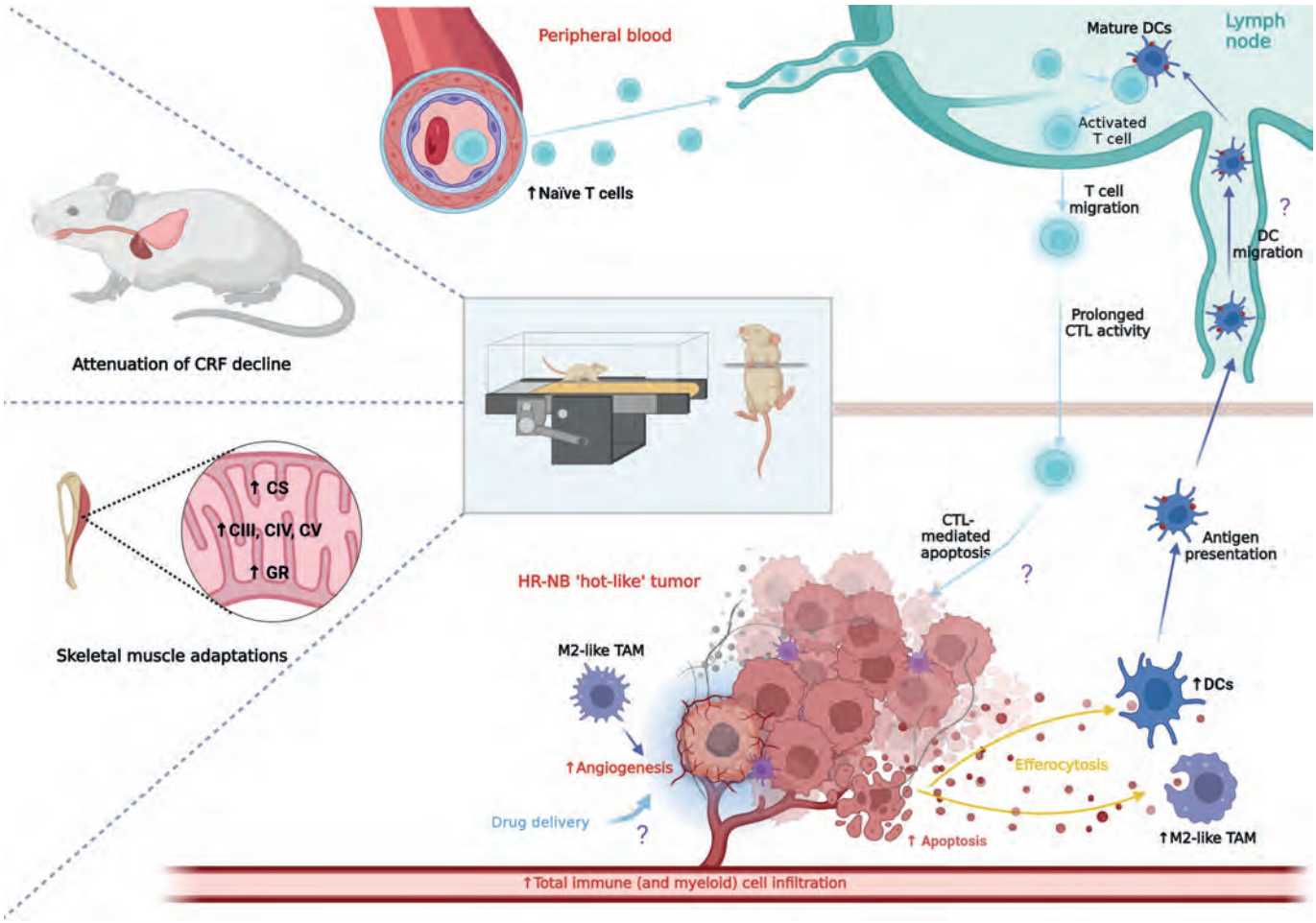
Background: Exercise might exert anti-tumoral effects in adult cancers but this question remains open in pediatric tumors, which frequently show a different biology compared to adult malignancies. We studied the effects of an exercise intervention on physical function, immune variables and tumoral response in a preclinical model of a highly aggressive pediatric cancer, high-risk neuroblastoma (HR-NB).

Methods: 6-8–week–old male mice with orthotopically-induced HR-NB were assigned to a control (N=13) or exercise (5-week combined [aerobic+resistance]) group (N=17). Outcomes included physical function (cardiorespiratory fitness [CRF] and muscle strength), as well as related muscle molecular indicators, blood and tumor immune cell and molecular variables, tumor progression, clinical severity, and survival.

Results: Exercise attenuated CRF decline (p=0.029 for the group-by-time interaction effect), which was accompanied by higher muscle levels of oxidative capacity (citrate synthase and respiratory chain complexes III, IV and V) and an indicator of antioxidant defense (glutathione reductase) in the intervention arm (all p≤0.001), as well as by higher levels of apoptosis (caspase-3, p=0.029) and angiogenesis (vascular endothelial growth factor receptor-2, p=0.012). The proportion of 'hot-like' (i.e., with viable immune infiltrates in flow cytometry analyses) tumors tended to be higher (p=0.0789) in the exercise group (76.9%, vs. 33.3% in control mice). Exercise also promoted greater total immune (p=0.045) and myeloid cell (p=0.049) infiltration within the 'hot' tumors, with a higher proportion of two myeloid cell subsets (CD11C+ [dendritic] cells [p=0.049] and M2-like tumor-associated macrophages [p=0.028]), yet with no significant changes in lymphoid infiltrates or in circulating immune cells or chemokines/cytokines. No training effect was found either for muscle strength or anabolic status, cancer progression (tumor weight and metastasis, tumor microenvironment), clinical severity, or survival.
Conclusions: Combined exercise appears as an effective strategy for attenuating physical function decline in a mouse model of HR-NB, also exerting some potential immune benefits within the tumor, which seem overall different from those previously reported in adult cancers.

Key words: cancer; pediatric cancer; exercise; immune function; immuno-oncology

**Correspondence to:*
Carmen Fiuza-Luces, PhD
Physical Activity and Health Research Group (PAHERG)
Instituto de Investigación Sanitaria Hospital Universitario 12 de Octubre ('imas12')
Centro de Actividades Ambulatorias (CAA), 7ª Planta, Bloque D
Av. de Córdoba s/n, 28041 Madrid, Spain
Phone: +34 91 779 2713; e-mail: cfiuza.imas12@h12o.es



Graphical abstract: Main effects of the exercise intervention and proposed potential mechanisms*. Abbreviations: CRF, cardiorespiratory fitness; CS, citrate synthase; CIII, CIV and CV, mitochondrial respiratory complex III, IV and V, respectively; CTL, cytotoxic T lymphocytes; DC, dendritic cells; HR-NB, high-risk neuroblastoma; TAM, tumor associated macrophages. * Naïve T cells (which were found in higher proportions in the blood of the exercise group than in control mice) constantly circulate through secondary lymphoid organs, where antigen encounter occurs. For a CTL response, antigen is brought to the lymph node via the lymphatic system by antigen presenting cells (which are typically DCs that mature after antigen acquisition in nonlymphoid tissues and migrate to the lymph node).

INTRODUCTION

A healthy lifestyle that includes regular physical activity is associated not only with a lower incidence of different types of adult cancers [1–4], but also with a lower risk of tumor recurrence or cancer-specific mortality [5–8]. Although most research has focused on the benefits of 'aerobic' physical activity (e.g., brisk walking, jogging), growing evidence also suggests that muscle resistance (or 'strength') exercise training – which together with aerobic activities is recommended by the World Health Organization (WHO) for health promotion in essentially all population groups [9] – can be associated with a lower risk of cancer and cancer-specific mortality [10]. In turn, epidemiological evidence is supported by preclinical research showing that regular aerobic exercise – typically voluntary (wheel) or forced (treadmill) running – could reduce tumor progression in rodents [11]. Although the different mechanisms underlying potential exercise antitumoral effects remain to be elucidated, an improvement in cancer immunosurveillance can be involved [12,13]. This is an important issue because the ability to evade immune destruction is one of the hallmarks of cancer [14], and immunotherapy (by means of stimulating natural immune defenses, e.g., using immunomodulators, monoclonal antibodies, oncolytic viruses or checkpoint inhibitors) is gaining importance in oncology [13]. On the other hand, an active lifestyle is also able to attenuate the side effects of cancer and its treatment, notably fatigue and decreased physical function [15]. Thus, exercise has been reported to improve cardiorespiratory fitness (CRF) and overall physical function in cancer survivors, thereby contributing to a better health-related quality of life (HRQoL) [16].

Most research in the field of exercise and cancer – especially preclinical studies – has focused on adult malignancies, with comparatively less research conducted in pediatric cancer. In this effect, toxicities associated to pediatric cancer treatment (chemotherapy/radiotherapy) are often persistent years after treatment and include among others harmful changes to growth, impaired cardiopulmonary function (e.g., cardiotoxicity) and alterations in body composition (excess abdominal adiposity, muscle weakness, poor bone health), together with low physical performance and difficulty of coping with activities of daily living, as well as cognitive decline (with subsequent impairment in academic, social, and professional performance later in life) [17] whereas physical exercise interventions can have the opposite effect. There is indeed meta-analytical evidence that, especially when supervised, exercise interventions during and/

or after pediatric cancer treatment are safe and can increase or preserve physical capacity (e.g., CRF) [18], cardiac function [18], functional mobility during daily life activities [19], muscle strength [20], physical activity levels [20], body mass index [20] and cognitive function [21], while reducing fatigue [20]. In fact, Pediatric Oncology Exercise Guidelines have been recently developed to promote physical activity among children and adolescents affected by cancer [22]. However, whether physical exercise can also impact pediatric tumor development is unknown, except for a preclinical study on tumor vascular modulation in the context of Ewing sarcoma (a pediatric bone and soft tissue cancer) [23]. In this effect, the biology of children's tumors might considerably differ from that of adult malignancies, as the former are overall characterized by a distinct (and usually lower) mutational burden, an embryonal (or very early) origin in many cases and/or a dysregulation of developmental pathways, together with a lower contribution of environmental factors [24]. In addition, pediatric tumor development might often take place in a moment of life when the immune system is not yet totally developed [25], which does not usually occur until age ~7-8 years [26].

The two main types of pediatric tumors are leukemias (30%) and solid tumors, with neuroblastoma (NB) representing the commonest extracranial solid malignancy [27] – accounting for >7% of all pediatric cancers [28,29] and 15% of related deaths [29–31]. Most NB cases are diagnosed between 0 and 4 years of age (median age ~19 months) [32], with less than 5% of affected children older than 10 years [28]. On the other hand, more than half of patients are above the age threshold – see below – for a worse prognosis [32]. The biological and clinical heterogeneity of NB fluctuates from spontaneous regression to metastatic dissemination depending on factors such as age at diagnosis (with age >15 months associated with worse outcomes [33]), tumor size and localization, histopathological classification, genetic abnormalities, and status of the *MYCN* proto-oncogene [34,35]. In this effect, high-risk (HR)-NB is typically associated with older age at diagnosis, unfavorable histology, metastatic phenotypes, as well as with some genetic modifications (DNA ploidy or specific segmental chromosomal aberrations) and *MYCN* gene amplification, with the prognosis for affected children being one of the poorest in pediatric cancer (i.e., 5-year survival <40-50% and <10% in patients with relapsed and refractory disease, respectively) [33,36,37]. Whether exercise could exert a certain antitumoral effect in the context of such an aggressive tumor as HR-NB, or at least attenuate the decline in physical function commonly observed in these patients [38], is an important clinical question. In this context, the use of animal models could help to provide valuable insights.

The purpose of this study was to analyze the effects of a combined (aerobic and resistance) exercise intervention on physical function, clinical evolution, immune (blood and tumor) variables, and tumor microenvironment in a preclinical mouse model of HR-NB. Our hypothesis is that exercise could induce some potential benefits, particularly at the intratumoral immune level.

METHODS

Mice

The study was approved by the Ethics Committee on Animal Experimentation and Welfare of the Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT; reference # ES280790000183, Madrid, Spain) and the Madrid Regional Department of Environment (reference # PROEX 036.7/21). All the study procedures were conducted in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines, as well as with the European (2010/63/UE Directive and European convention ETS 123) and Spanish (Law 6/2013 and Royal Decree 53/2013) regulations on animal protection in scientific research.

Wild-type 129/SvJ 6-8–week–old male mice were purchased from Vivotecnia (National Cancer Institute; Frederick, MD). They were bred and housed in the animal facility of CIEMAT under controlled conditions of temperature (20±2ºC) and humidity (55±10%) with adequate environmental enrichment (nestlets), ventilation and constant 12-hour light/dark cycles, and with food and water provided ad libitum. They were used to generate an orthotopic murine model of HR-NB, as described below. All efforts were made to minimize animal suffering, which implied minimizing blood sampling and allowing a 3-week recovery period from surgery until baseline assessments.

Tumors

Cell cultures and preparation of neuroblastoma sphere-forming cells

The 36769-cell line kindly donated by Prof. Chesler (Royal Marsden NHS Foundation Trust; London, UK) was derived from a tumor mass developed in a TH-*MYCN*-129X1/SvJ transgenic mouse, which is a spontaneous model with *MYCN* amplification that recapitulates the genetic, histopathological, and clinical features of HR-NB [39]. These cells were maintained as described in previous research [40], using Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12) (Gibco; Carlsbad, CA) supplemented with 1% penicillin/streptomycin (Gibco), 1xB-27 without vitamin A (Gibco), 20 ng/mL murine epidermal growth factor (R&D Systems; Minneapolis, MN) and 40 ng/mL murine fibroblast growth factor (R&D Systems) at 37ºC with 5% CO_2. The culture medium was renewed every 72 hours, and spheres were seed and expanded at 1/6 of initial concentration (for 8-10 days) before implantation. In the log phase of growth, they were transferred from the culture flask to a Falcon® tube and centrifuged at 900 g for 5 minutes. The spheres were incubated in trypsin (TripLE Express, Life Technologies; Carlsbad, CA) for 3-5 minutes at 37ºC. Thereafter, the spheres were carefully mechanically-dissociated into single cells by pipetting, culture medium was added, the solution was centrifuged (1500 rpm, 5 minutes) and finally the cells were counted and prepared for later inoculation.

Implantation in mice

A single suspension of $1 \cdot 10^5$ cells in 30 µL of unsupplemented DMEM/F12 was orthotopically inoculated into the left adrenal gland (which is where 40% of human NB tumors arise [41]) of the mice. This was done with an intraperitoneal approach through supraumbilical transverse laparotomy under general anesthesia – induced by an intraperitoneal injection of ketamine and dexmedetomidine (50 ng/g), using atipamezole (2.5 ng/g) as an anesthesia reversal agent.

Study design

During the 3-week period following surgery, the clinical

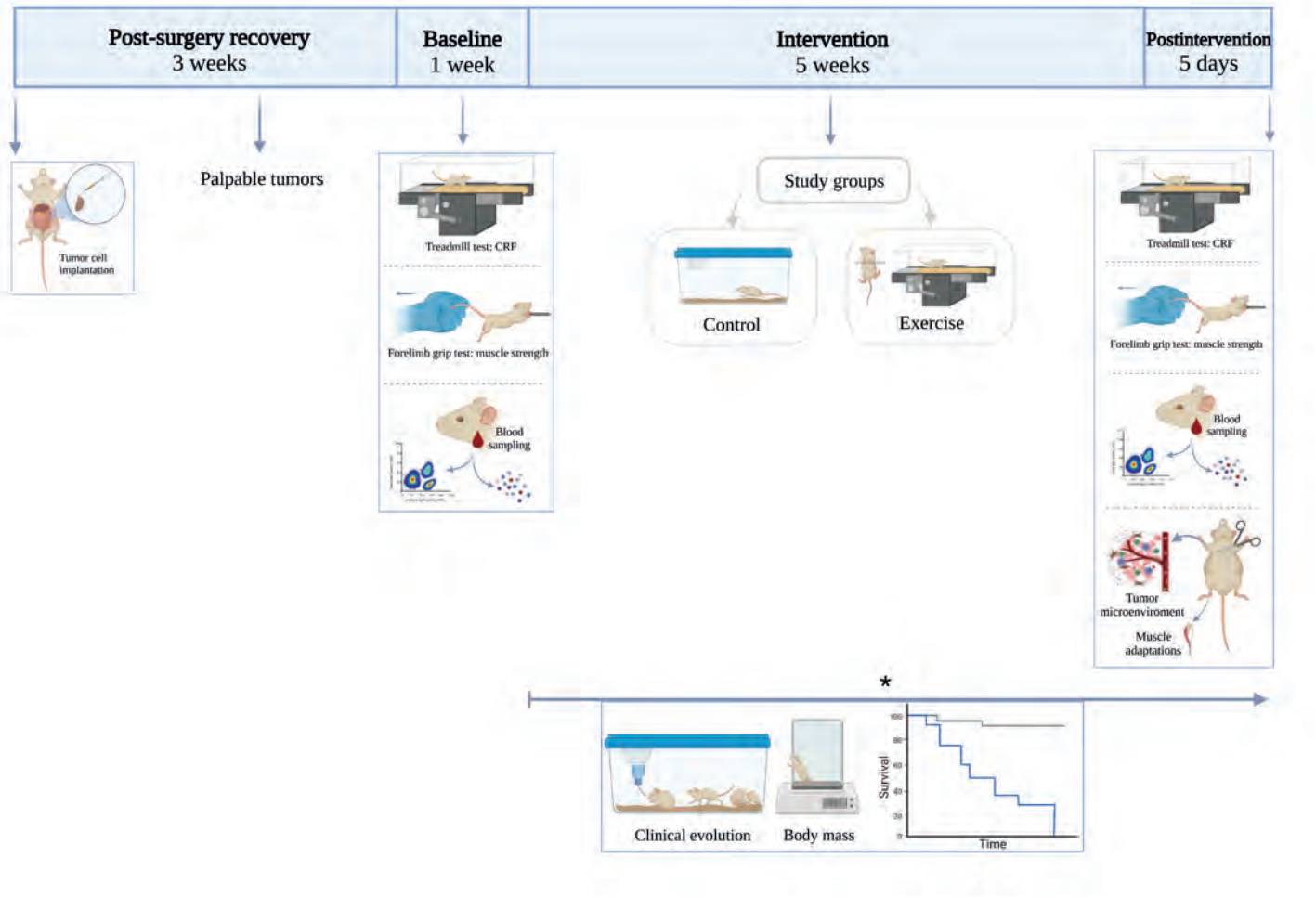


Figure 1. Study design. * Clinical evolution, body mass and survival (Kaplan Meyer curve) were determined from the start of the exercise intervention until death or end of the study (i.e., end of the 5-day postintervention phase).

evolution of the disease was evaluated every 2 days (Figure 1). Thus, mice were weighed and their posture, spontaneous activity, hair and scar were controlled to ascertain they had recovered from surgery. The post-surgery recovery phase was followed by the familiarization phase.

Familiarization

Mice were familiarized with the apparatuses and types of exercise involved in the physical evaluation tests – treadmill running (Harvard Apparatus, Panlab; Barcelona, Spain) and isometric force testing (Harvard Apparatus) [42]. This allowed the animals to gradually adapt to the new environmental conditions, and thus to reduce their stress levels while minimizing measurement error during physical testing. It also allowed us to ensure that the wound healing process had ended so that animals would be able to do the familiarization exercises (see below) and subsequent baseline physical tests safely.

The familiarization with the treadmill was performed on 3 separate days, by applying a gradual increase in running time, as well as in treadmill velocity and inclination (starting with 5 minutes at 5 cm/s and 5% inclination on the first day, and ending with a 12-minute run at 10 cm/s and 15% inclination on the third day, in all cases with an electrical stimulation of 0.1 mA). A total of 5 individual treadmills were used but each mouse was solely trained and tested with the same apparatus. Mice were also familiarized with the isometric force test, simulating the forelimb grip strength test on 3 separate days (see below).

Timing of physical tests and tissue/blood sampling

All the mice underwent the same physical tests (for determination of CRF and forelimb muscle strength) before (baseline) and after the intervention period. Blood sampling from the submandibular vein (200 µL) was also performed at baseline (24 hours before the first battery of physical tests) and postintervention (48 hours after the last training session). Peripheral blood was drawn into EDTA or serum separator tubes (BD Microtainer®, Becton Dickinson; Franklin Lakes, NJ) in order to preserve the sample for immunophenotype analysis or to obtain (after centrifugation [1600 rpm, 5 minutes, twice]) a serum sample for analysis of cytokines/chemokines.

Mice were sacrificed by cervical dislocation after postintervention blood sampling and their tumors were excised, weighed and measured in 2 perpendicular dimensions using a vernier caliper for tumor volume calculations, and then divided into 3 pieces. One piece was fixed in 4% paraformaldehyde and embedded in paraffin prior to immunohistochemical studies. Another one was preserved in Hank's Balanced Salt Solution 1X (HBSS9) (Gibco) and appropriately mechanically-processed and enzymatically digested in RPMI 1640 medium with 1 mg/mL of collagenase D (Roche Diagnostics Corporation; Indianapolis, IN) to detect intratumoral immune cell infiltrates by flow cytometry. A third piece was frozen in liquid nitrogen and conserved at -80ºC until gene expression analysis using real-time quantitative polymerase chain reaction (qRT-PCR). In addition, several tissues (kidneys, liver, gut, pancreas, spleen,

lung, brain) were extracted and fixed in formalin, excised and embedded in paraffin for hematoxylin and eosin (H&E) staining to detect potential metastasis. Finally, skeletal muscle tissue (tibialis anterior) was dissected, immediately frozen in liquid nitrogen and stored at -80ºC until molecular analysis of muscle adaptations (see further below) by western blotting.

Group assignment

Mice were pair-matched based on the results of the treadmill tests. Thus, each two mice showing the closest values of CRF were matched together and randomly assigned to either an exercise or non-exercise (control) group. The former group performed the exercise training intervention that is described below whereas mice in the control group were allowed to freely move in the cage but only walked on the treadmill for 5 minutes (speed =5 cm/s, inclination =0%) once a week.

Intervention

The exercise intervention was designed following our previous experience with other mouse models [42,43], with slight modifications based on the singularities of the HR-NB model used in the present study. The program (4 days/week [Monday to Thursday], 40-60 minutes per day) lasted 5 weeks in total.

Aerobic training

All the sessions were performed between 09.00 am and 13.00 pm and included an aerobic (treadmill) training component, starting and ending with a warm-up and cool-down period, respectively (5-8 minutes at 35-40% of the maximal velocity [Vmax] obtained during the baseline incremental treadmill test, 0-15% inclination). For the main part, exercise duration, treadmill speed, and inclination were gradually increased. Thus, the program began at low workloads on the first week (25-33 minutes at 50-55% of Vmax, 5% inclination) and ended with moderate workloads (30-40 minutes, 70-75% of Vmax, and 15% inclination). In order to minimize animal discomfort, only gentle back touching (with no electrical stimulation) was used during each training session.

Resistance training

The aerobic training phase was followed by resistance training on 3 days/week (Monday, Wednesday, and Thursday). After several attempts with different resistance exercise modalities [44], we considered that the best option for this mouse model consisted of having the animals grasp a cloth hanger (maintained at 40 cm above a layer of bedding to cushion the falls) while only using the two forepaws for as long as possible (6 attempts interspersed with 10-minute rest periods).

Outcomes

Cardiorespiratory fitness

An incremental treadmill test was used to determine the CRF of the mice following a previously described protocol with slight modifications [42]. Mice were subjected to an initial warm-up period (5 minutes at 5 cm/s and 15% inclination; followed by 5 minutes at 8 cm/s and 15% inclination) after which treadmill speed was increased by 2 cm/s every 2 min (while inclination kept constant at 15%) until exhaustion – that is, until the mice spent more than 5 seconds on the electric grid at the back of the treadmill and were unable to continue running. Electrical stimulation was kept at 0.2 mA/1 Hz during the test. The total distance (meters) achieved by each mouse was recorded as the CRF (or 'maximal aerobic capacity').

Forelimb grip strength

Muscle strength was measured as the maximum force (g) exerted by the mice before losing grip, using an isometric force transducer (Harvard Apparatus) [42] on the day before the treadmill test. Each mouse took the test 3 times with a 5-minute rest period between them, and the best reading was recorded as the maximal grip strength.

Skeletal muscle measures

Frozen skeletal muscle tissues were processed as described elsewhere [45], with ice-cold 10 mM Tris-HCl pH 7.6, containing 150 mM NaCl, 1 mM EDTA, 1% Triton™ X-100 and a protease and phosphatase inhibitor cocktail (Roche Diagnostics Corporation; Indianapolis, IN) 1:10 (weight:volume) in a potter homogenizer. Protein concentration was determined with the Pierce® BCA protein assay kit (Thermo Fisher Scientific; Waltham, MA) according to manufacturers' instructions.

Tibialis anterior homogenates (20-40 µg) were analyzed through western blotting to determine indicators of aerobic metabolism (citrate synthase [CS] and mitochondrial respiratory chain subunits), anabolic status (ratio of phosphorylated/activated ribosomal protein p70 S6 kinase [pP70S6K] to total P70S6K), and antioxidant defense (catalase, glutathione reductase, mitochondrial superoxide dismutase). Briefly, sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed on a 7.5-12.5% separation gel. Resolved proteins were transferred to a polyvinylidene difluoride (PVDF) membrane, blocked with a solution of Tris-HCl with Tween 20 0.1% (TBST) with 5% skimmed milk or bovine serum albumin and incubated with primary and peroxidase-conjugated secondary antibodies (Supplemental file 1), which were immunodetected using the ECL Prime Western Blotting Detection Reagent (Amersham GE Healthcare; Little Chalfont, UK) in the image analyzer ChemiDocTM MP Imaging System (Bio-Rad; Hercules, CA). Band densities were determined by densitometric scanning (Image LabTM Software, version 5.0., Bio-Rad). Total protein load per lane was determined with Coomassie Blue staining of the PVDF membranes [46].

Survival

Survival was evaluated by counting the time (number of days) elapsed from the start of the intervention until 'spontaneous' death (i.e., attributable to the tumor) or end of the study (with the latter corresponding to the end of the 5-day postintervention phase, as previously shown in Figure 1). In addition, based on ethical reasons those mice not dying before the end of the study were sacrificed if showing signs of very poor health status or significant suffering (i.e., severe hunching impairing movement, lack of spontaneous mobility, and facial expression of pain).

Clinical evolution

Mouse body mass was measured and disease severity scored, respectively, in each mouse by the same researcher every weekday during the same (aforementioned) period as survival. A total score was computed on a 0 (absence) to 3 (maximal severity) scale by adding individual scores for the following variables: posture (0 [normal], 0.5 [hunching noted only at rest]) or 1 [severe hunching impairing movement]); fur texture

(0 [normal], 0.5 [mild-to-moderate ruffling], or 1 [severe ruffling/poor grooming]); and activity (0 [normal], 0.5 [mild-to-moderately decreased], or 1 [stationary unless stimulated]) (adapted from [43]).

Metastasis

Representative samples of tumors and other organs (kidneys, liver, gut, pancreas, spleen, lung, and brain) were excised and included in formalin-fixed paraffin-embedded (FFPE) blocks. FFPE samples were sectioned at a 3 μm thickness with a microtome, mounted onto microscope slides and stained with H&E. Morphological evaluation by senior pathologists (FR, SZ) was performed on representative sections from primary tumor and mice organs in order to detect metastatic deposits.

Tumor volume

Tumor volume was calculated upon sacrifice with a digital caliper in two orthogonal dimensions using the following formula: volume (mm^3) =1/6π x length (mm) x $width^2$ (mm^2), where tumor length was the greatest dimension of the tumor with the other dimension (at a 90º angle) being taken as the width. Tumors were also weighted upon sacrifice (ME303T/00, Mettler Toledo; Barcelona, Spain).

Tumor immunohistochemistry

For each case, FFPE tumor samples were assessed for markers of apoptosis, cell cycle control, and proliferation. Immunohistochemistry analyses (antibodies described in detail in Supplemental file 2) were performed in 3 μm sections of FFPE tumor tissue using coated microscope slides (FLEX IHC Microscope Slides; Dako Omnis, Agilent; Glostrup, Denmark). The slides were incubated for 1 hour at 56 ºC to allow the tumor section to be perfectly adhered before staining. Immunohistochemistry analyses were performed automatically on the Autostainer Link 48 (Agilent) using the EnVision™ + System-HRP (DAB) kit (Dako Omnis) following the manufacturer's instructions.

In some cases (cleaved caspase-3, Ki-67, vascular endothelial growth factor receptor 2 [VEGFR2], and Factor VIII), it was necessary to include an intermediate step in order to amplify the signal thought an additional 20-minute incubation with EnVision™ FLEX + Rabbit. Thereafter, HRP staining was visualized with 3,3'-diaminobenzidine tetrahydrochloride in organic solvent (DAB) as chromogen, adding EnVision™ FLEX DAB + Chromogen (Dako Omnis) during 10 minutes, and using hematoxylin reagent (EnVision™ FLEX Hematoxylin, Dako Omnis) for counterstaining during 5 minutes. When the staining was ended, the slides were mounted on the automatic CoverStainer (Dako Omnis). Those slides that were only stained with secondary antibodies were used as negative controls.

Visualization of digital slides and image processing was performed by the Philips system (IntelliSite Image Management System; Philips; Best, NL). The stained sections were evaluated semi-quantitatively by two independent pathologists (FR, SZ) blinded to group assignment. Expression of Ki-67, histone deacetylase 3 and cleaved caspase-3 were evaluated in tumor cells as % of stained cells. Nuclear staining was required for evaluation. The expression levels of VGEFR2 were determined as the % of vessels with positive endothelial cells for VEGFR2 relative to the total number of vessels per a 20x field (with total vessel number [average of 3 measurements] identified with Von Willebrand Factor-related antigen [commonly known as 'Factor VIII'] staining).

Gene expression in tumor microenvironment

A tumor piece (30 μg) previously preserved at -80ºC after dissection was mechanically disrupted and processed for RNA isolation (RNeasy Plus Mini Kit, Qiagen Inc.; Hilden, Germany) and quantification was done using NanoDrop 100 (Thermo Fisher Scientific). cDNA was generated from 1 μg of RNA and reverse transcribed using SuperScript™ VILO™ cDNA Synthesis Kit (Invitrogen, Thermo Fisher Scientific). The following genes were studied using specific murine TaqMan assays (all from Applied Biosystems™; Foster City, CA) for qRT-PCR analysis (7500 Real-Time PCR System, Applied Biosystems™) using glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*; Mm99999915_g1), as the housekeeping gene: chemokine (C-C motif) ligand 2 (*Ccl2*; Mm00441242_m1) and 3 (*Ccl3*; Mm00441259_g1), intercellular adhesion molecule 1 (*Icam1*; Mm00516023_m1), interferon beta 1 (*Ifnb1*; Mm00439552_s1) and gamma (*Ifng*; Mm01168134_m1), interleukin 15 (*Il15*; Mm00434210_m1), metallopeptidase 9 (*Mmp9*; Mm00442991_m1), tumor growth factor beta 1 (*Tgfb1*; Mm01178820_m1), tumor necrosis factor (*Tnf*; Mm00443258_m1), vascular cell adhesion protein 1 (*Vcam1*; Mm01320970_m1), and vascular endothelial growth factor A (*Vegfa*; Mm01281449_m1) and R1 (*Vegfr1*; Mm00438980_m1).

Tumor samples were analyzed in duplicate using 7500 Real-Time PCR software v2.0.6 (Applied Biosystems™). The formula $2^{-\Delta\Delta Ct}$ was used to calculate the relative expression ratio in experimental groups, where all values were normalized to *Gapdh*. Data are presented with respect to non-exercise (control) group.

Immune analyses in tumors and blood

Before analyzing immune infiltrated cells, tumor mass was carefully washed with HBSS 1X and mechanically processed prior to enzymatic digestion with 1 mg/mL of collagenase D (Roche; Catalog #11088858001). Tumor cell suspension was counted by trypan blue and $5 \cdot 10^5$ alive cells were used for immune cell phenotype analysis.

Peripheral and tumor immune cells were studied with flow cytometry. Homogenates of tumor tissue and fresh peripheral blood samples were incubated using appropriate combinations of monoclonal antibodies (Supplemental file 3) at 4ºC in the dark for 30 minutes. Red blood cells were lysed using QuickLysis buffer (Cytognos S.L.; Santa Marta de Tormes, Spain) for 30 minutes at room temperature in darkness. A minimum of 20,000 events were acquired in a FacsCanto II cytometer (BD Biosciences; San Jose, CA) and analyzed using FacsDiva software v6.1.2 (BD Biosciences). Details on the procedures used from the moment of tumor sample collection until actual immune cell phenotyping of tumor infiltrates with flow cytometry are shown in Supplemental file 4.

We determined CD45+ cells (commonly known as 'leukocytes'), discerning between myeloid components (macrophages and myeloid-derived suppressor cells [MDSC] [CD11B+], and dendritic cells [CD11C+]), on the one hand, and lymphoid components (T [CD3+], B [B220+], and natural killer [NK] cells [NK1.1+]), on the other. The different T lymphocyte subtypes (helper [CD4+] and cytotoxic T cells

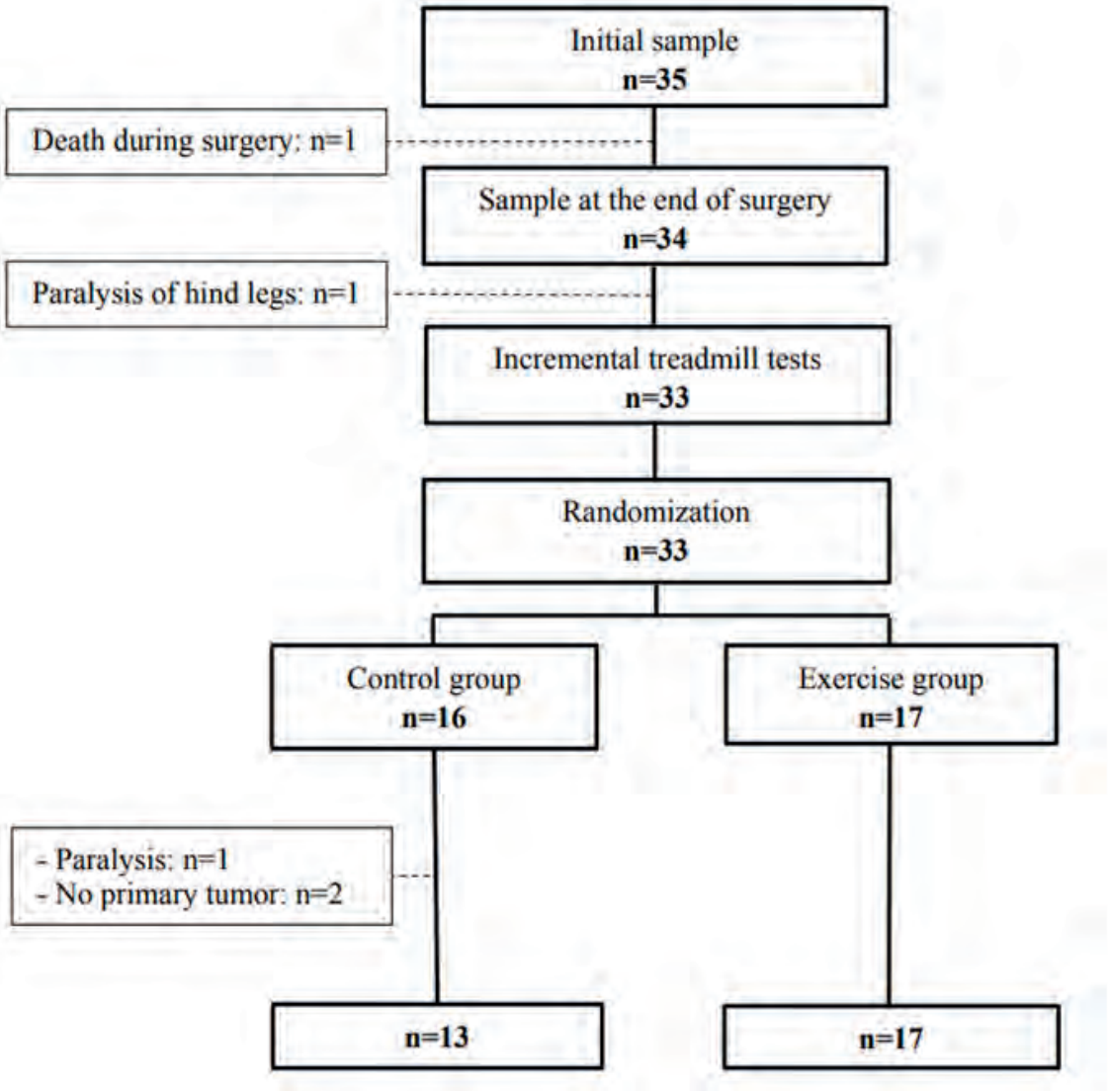


Figure 2. Study flow diagram.

[CD8+]) were also analyzed. T-cell differentiation was defined as the proportion of naïve (CD4+/CD8+ CD62L+ CD44–) and central (CD4+/CD8+ CD62L+ CD44+), effector (CD4+/CD8+ CD62L– CD44+) or terminally differentiated effector memory (CD4+/CD8+ CD62L– CD44–), respectively, within the total CD4 or CD8 T-cell population. Recent (OX40+, 4-1BB+) and sustained lymphocyte activation (programmed cell death 1 [PD1]+), lymphocyte-activation gene 3 [LAG3]+, and T-cell immunoglobulin and mucin domain-containing protein 3 [TIM]3+) states were also analyzed. The two major subsets of MDSC (CD45+ CD11B+), such as granulocytic (CD11B+ Ly6G+ Ly6Clow) and monocytic MDSC (CD11B+ Ly6G– Ly6Chigh) cells, and M1-like (CD11B+ Ly6G– Ly6C– CD206–) and M2-like (CD11B+ Ly6G– Ly6C– CD206+) macrophages were also included in the analysis. Finally, dendritic cells (DCs) were analyzed including the plasmacytoid (CD45+ CD11C+ CD123+) and myeloid (also known as 'conventional'; CD45+ CD11C+ CD123–) subsets, respectively.

The levels of the following cytokines/chemokines were determined in serum samples at baseline and postintervention using a multiplex Mouse Cytokine Panel (Merck Life Science; Madrid, Spain) based on the Lumine xMAP® technology on a MAGPIX® instrument (EMD Millipore Corporation; Burlington, MA) according to the manufacturers' instructions: granulocyte-macrophage colony-stimulating factor (GM-CSF), IFNβ1, IFNγ, IL2, IL6, IL15, macrophage inflammatory protein (MIP)1α, monocyte chemoattractant protein (MCP)1, TNFα, and VEGF.

Statistical analyses

A two-factor (group, time), ANOVA with repeated measures on time was used to determine the effects of the exercise intervention on those outcomes that were measured both at baseline and postintervention (i.e., CRF, forelimb grip strength, and all blood variables [i.e., immune cell phenotypes and cytokines/ chemokines]). The Mann Whitney U test was used for unpaired comparisons in those outcomes that were measured only at postintervention (i.e., all skeletal muscle and tumor-related variables). The χ^2 test (or Fisher's exact test if >20% of the cells in the cross-table had an expected frequency <5) was used for between-group comparisons of the proportion of metastases and 'hot tumors', and the Kaplan-Meier analysis (right censored) was used for survival comparisons. On the other hand, whether the pattern of missing data for the different measurements

Table 1. Cardiorespiratory fitness (CRF) and muscle (forelimb) grip strength by group.

Outcome	Group	Baseline	Postintervention	% change	Group (p)	Time (p)	Group by time (p)
CRF (meters)	Control	653 ± 80	193 ± 35	-70 ± 56	0.677	**<0.001**	**0.039**
	Exercise	595 ± 72	320 ± 60	-46 ± 17			
Grip strength (grams)	Control	172 ± 6	149 ± 7	-13 ± 17	0.996	**0.020**	0.792
	Exercise	174 ± 11	151 ± 7	-13 ± 36			

Data are mean ± SEM. Total N with complete data =11 (control) and 12 (exercise).

followed each of three possibilities was also analyzed [47]: a) missing completely at random (i.e., no relationship between the missingness of the data and any values, observed or missing); b) missing at random (i.e., existence of a systematic relationship between the propensity of missing values and the observed data, but not the missing data); and c) missing not at random (also known as 'non-ignorable missingness', i.e., the data are neither missing completely at random nor missing at random, with the value of the missing variable related to the reason it is missing) .

RESULTS

The initial number of mice before tumor cell inoculation was 35 (Figure 2). One of them died during surgery, another one was excluded as its hind legs were paralyzed before starting the familiarization phase (morbidity associated with the technique = 3%), two did not present the primary tumor into the left para-adrenal space despite showing several masses located in other parts of the body (e.g., abdominal region), and another one showed paralysis in its hind legs during the intervention phase. These mice were not included in the analysis of the results. Therefore, the efficiency of engraftment of the tumor cell line was 94%. The final number of mice completing the study was 13 and 17 in the control and exercise group, respectively.

On the other hand, despite the lack of data for analyses from several mice (as detailed in the Tables and Figures below), the missing data for the different outcomes followed a missing-completely-at-random pattern, which rules out, at least partly, the possibility of inherent bias.

Physical fitness

Physical capacity declined throughout the study in both groups, yet with a significantly attenuation of CRF decline in the exercise group compared with control mice (p=0.039 for the group by time interaction effect; Table 1). No changes were found, however, in muscle grip strength.

Skeletal muscle adaptations to training

Higher levels (all p≤0.001) of several markers of muscle aerobic adaptations (i.e., CS levels, as well as those of respiratory chain complexes III, IV and V) and antioxidant defense (glutathione reductase) were found in the exercise group compared with control mice, although no differences were found for muscle anabolic status indicators (pP70S6K/P70S6K ratio) (Figure 3).

Clinical data

Survival

No significant between-group differences were found in survival (Figure 4), with median values of 28 days (range 11

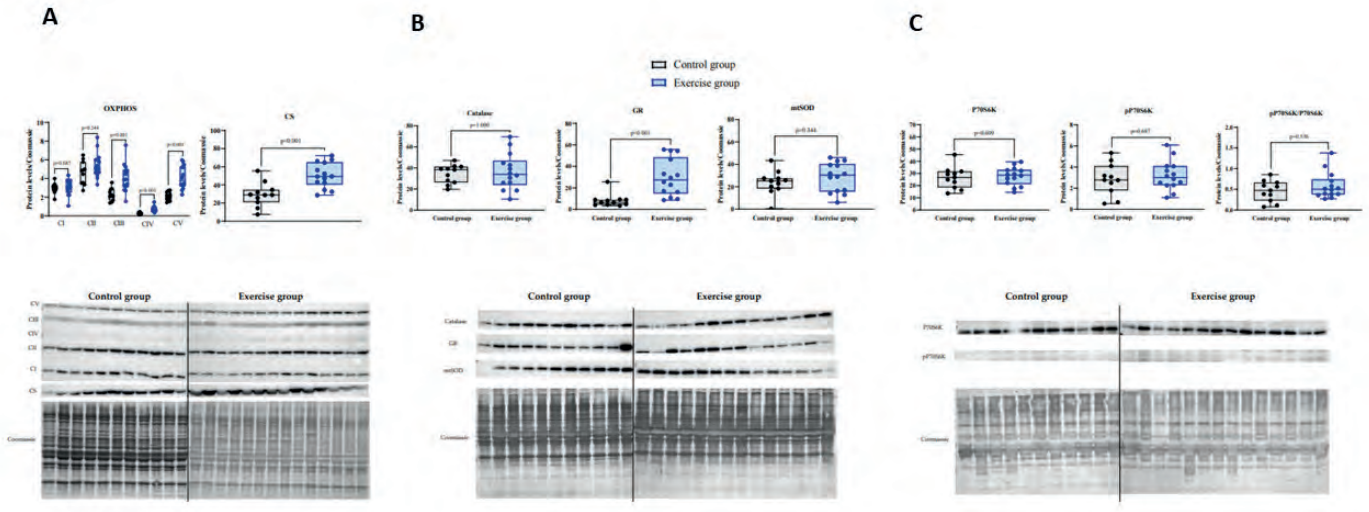


Figure 3. Markers (western blot results) of exercise training adaptations in total skeletal muscle (tibialis anterior) homogenates by group (with total protein content per lane determined by Coomassie Blue 280 staining used as loading control). Data are mean ± SEM and individual data values (N with data = 11 [10-11 for panel B] and 14 in the control and exercise groups, respectively) and p-values for between-group comparisons are also shown. Panel A. Oxidative phosphorylation (OXPHOS) components (mitochondrial respiratory complexes I to V [CI to CV]) and citrate synthase (CS). Of note, the commercial cocktail of antibodies (Supplementary file 1) been used allows to determine all five complexes CI to CV simultaneously. Panel B. Antioxidant defense (catalase, glutathione reductase [GR] and mitochondrial superoxide dismutase [mtSOD]). Panel C. Proteins involved in anabolic status (phosphorylated/activated ribosomal p70 S6 kinase [Thr389] [pP70S6K] and total ribosomal protein p70 S6 kinase [P70S6K]). The ratio of pP70S6K to P70S6K reflects the anabolic status in the analyzed muscle.

to 37) and 25 days (3 to 37) in the exercise and control group, respectively

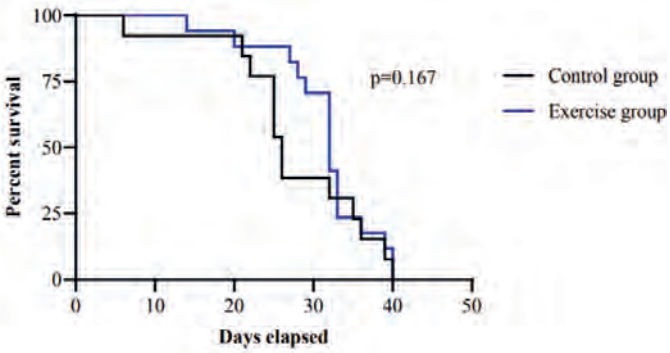


Figure 4. Survival estimates by group (with the p-value for the between-group comparison also shown). Total N with data =13 and 17 for the control and exercise groups, respectively. Survival was evaluated by counting the time (number of days) elapsed from the start of the intervention until death or end of the study (i.e., end of the 5-day postintervention phase previously shown in Figure 1) and therefore the timeframe before the start of intervention (post-surgery recovery phase and baseline measurements, previously explained in Figure 1) is not shown.

Clinical evolution

During the first week after the tumor cell inoculation, a deterioration in health status was noted in all the mice, as reflected by an increase in all individual scores (hair, posture, spontaneous activity). During the following ~2 weeks, however, there was a progressive improvement in health status, with all animals reaching a good recovery level in the different disease evolution parameters before beginning the intervention phase. The same trend was observed for body mass during this period of time (data not shown). From the start of intervention to the end of it, no significant group by time interaction effect (all p>0.1) was found for body mass (Figure 5A) or clinical severity (individual/total) scores (Figure 5B).

Tumor development and metastases

Tumor weight and volume

Tumors were palpable ~2 weeks after tumor cell inoculation and thus ~1 week before baseline assessments. Mean tumor volume and weight did not significantly differ between groups at sacrifice (Figure 6).

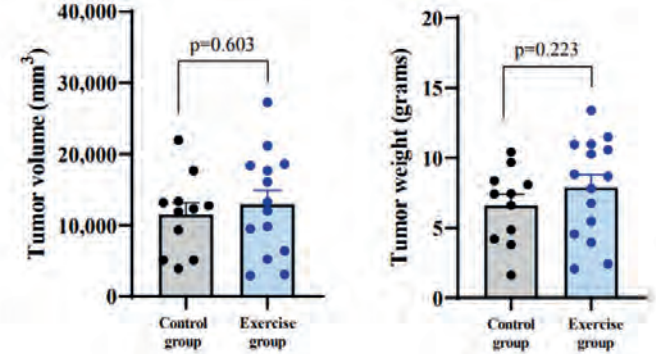


Figure 6. Tumor volume and weight at sacrifice by group. Data are mean ± SEM. Individual data values and p-values for between-group comparisons are also shown. N with = 11 and 14 (control and exercise group, respectively).

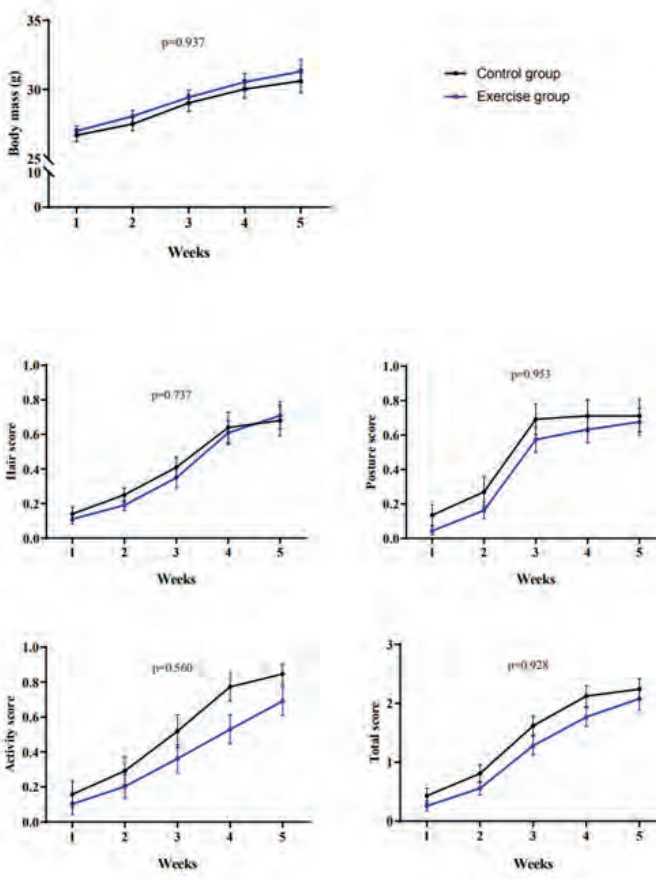


Figure 5. A. Body mass evolution by group from the start to the end of the intervention phase, respectively. No significant group by time effect (two-factor [group, time] ANOVA with repeated measures on time) was found. B) Daily clinical disease progression (individual and total disease severity scores, where a higher score denotes a higher severity) by group from the start to the end of the intervention, respectively. No significant group by time effect (two-factor [group, time] ANOVA with repeated measures on time) was found for individual (hair, posture, activity) or total scores. Data are mean ±SEM, with p-values for group by time interaction effect shown. N with complete data = 13 and 17 (control and exercise group, respectively).

Metastasis

No significant between-group differences were found for the prevalence of metastatic lesions (Table 2). The tissues most commonly affected by metastasis were the spleen and the liver, accounting for 83% of all metastases for both groups combined, with lung and pancreas metastases (in one mouse of the exercise and control group, respectively), accounting for the remainder of metastases (Figure 7).

Table 2. Metastasis analysis by group and analyzed organ.
N with complete data of = 5 (control) and 10 (exercise). The p-value corresponds to the Fisher exact text.

Organ	Group	Mice with metastasis (%)	p-value
Spleen	Control	60%	0.580
	Exercise	33%	
Liver	Control	33%	0.560
	Exercise	20%	
Pancreas	Control	33%	0.095
	Exercise	0%	
Lung	Control	0%	1.000
	Exercise	13%	
Brain	Control	0%	1.000
	Exercise	0%	
Gut	Control	0%	1.000
	Exercise	0%	
Kidneys	Control	0%	1.000
	Exercise	0%	

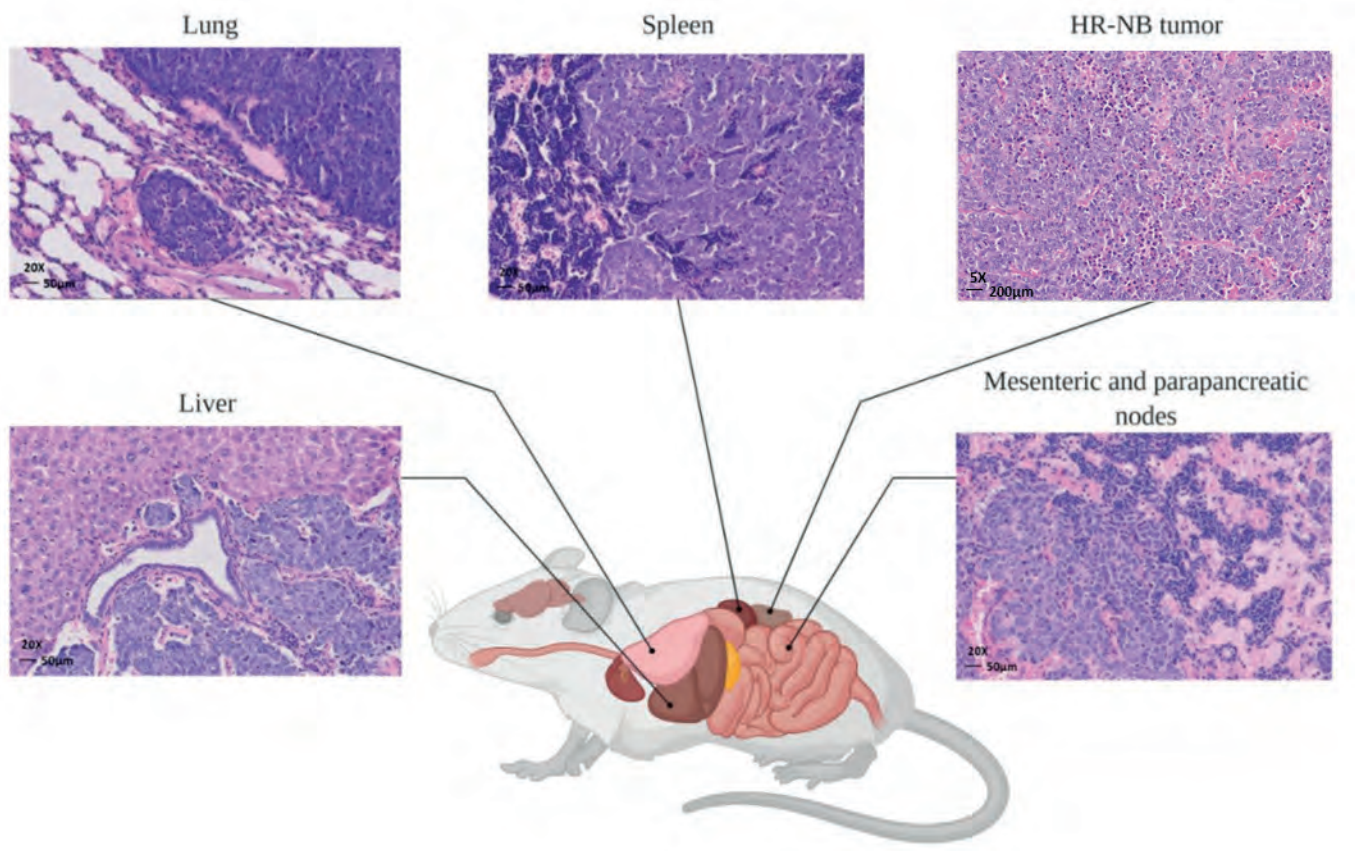


Figure 7. Representative images from hematoxylin and eosin staining of the most commonly affected tissues with metastatic lesions (lung, spleen, liver and pancreas) and the tumor.

Intratumoral studies

Immunohistochemical analyses

H&E staining of the tumors showed a significantly higher proportion of positive cells for the markers of apoptosis and angiogenesis that we studied, caspase-3 (p=0.029) and VEGFR2 (p=0.012), respectively, in exercised mice compared with controls (Figure 8) – although no between-group differences were found in the total vessel count (26.2 ± 2.2 and 26.8 ± 3.9 per x20 field, respectively, p=0.789). Yet, no significant between-group differences were found for Ki67 (p=0.240; with the vast majority of cells [≥96%] positive for this marker of proliferation in both groups) or histone 3 deacetylase (16.0 ± 2.8% versus 11.9 ± 1.8% for control and exercise groups, respectively; p=0.438).

Gene expression associated with the tumor microenvironment

No significant between-group differences were found for gene expression associated with the tumor microenvironment, although a trend towards higher values of *Tgfb1* expression levels and lower values of *Ccl2* expression levels were observed in the exercise group (p=0.067 and p=0.072, respectively) (Figure 9).

Immune cell phenotyping

Peripheral blood. Compared to control mice, the exercise group showed a significant increase from baseline to postintervention in the proportion of CD4 naïve T cells compared to controls (p=0.050 for the group by time intervention effect) and a similar (albeit nonsignificant) trend was found for the proportion of CD4 T cells expressing markers of recent (OX40) T cell activation (p=0.093) and central memory CD4 T cells (p=0.072) (Table 3). No significant differences were shown between the groups if data expressed as cell counts (data not shown).

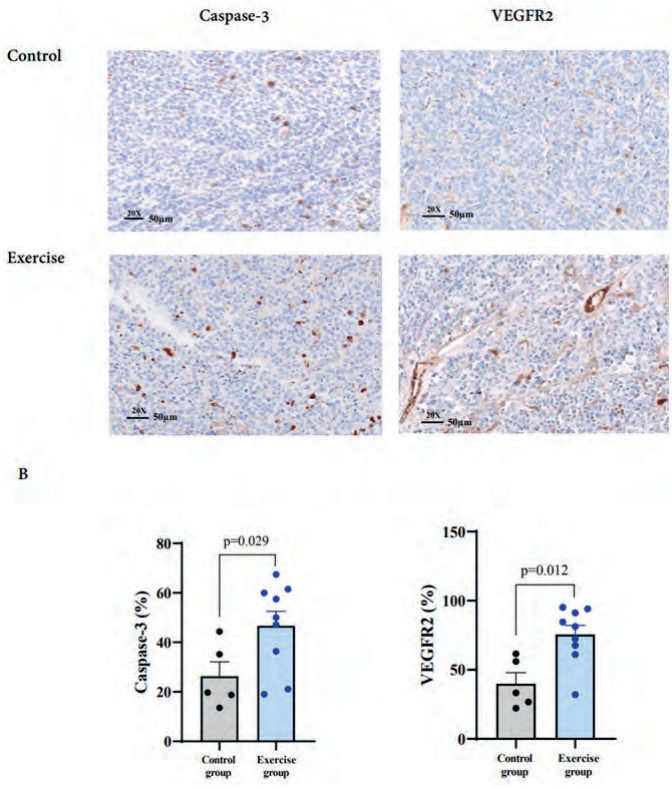


Figure 8. Immunohistochemistry analyses of markers of apoptosis (cleaved caspase 3) or angiogenesis (vascular endothelial growth factor receptor 2 [VEGFR2]) by group. (A) Representative images showing higher caspase-3 and VEGFR2 expression levels, respectively, in a mouse from the exercise group compared with a control. (B) Mean ± SEM values (together with individual data) expressed as % of positive cells, of these two markers in each group (with p-values for between-group comparisons also shown). N with data = 5 and 9 (control and exercise group, respectively).

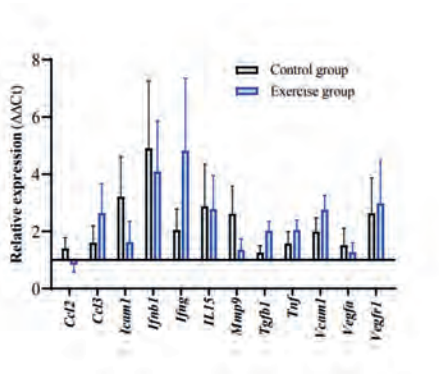


Figure 9. Gene expression analysis (real-time quantitative polymerase chain reaction) in homogenized tumors by group. Abbreviations: *Ccl2*, chemokine (C-C motif) ligand 2; *Ccl3*, chemokine (C-C motif) ligand 3; *Icam1*, intercellular adhesion molecule 1; Ifnb, interferon beta; *Ifng*, interferon gamma; *Il15*, interleukin 15; *Mmp9*, metallopeptidase 9; *Tgfb1*, transforming growth factor beta 1; *Tnf*a, tumor necrosis factor alpha; *Vcam1*, vascular cell adhesion protein 1; *Vegfa*, vascular endothelial growth factor-a; and *Vegfr1*, vascular endothelial growth factor receptor 1. Data are mean ± SEM with relative gene expression calculated as mRNA intratumoral levels normalized to those of the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase. No significant between-group differences were found (all p>0.1), although a trend to higher values of *Tgfb1* and lower values of *Ccl2* transcripts in the exercise group (p=0.067 and p=0.072, respectively) were observed. N with data = 11 and 14 (control and exercise group, respectively).

Tumors

In flow cytometry analyses, the proportion of 'hot' tumors tended to be higher (p=0.079 for Fisher exact test) in the exercise group (76.9% [10 of 13]) compared with control mice (33.3% [3 of 9]) (examples shown in Supplemental file 5). In turn, within 'hot' tumors, those from exercised mice showed significantly higher levels of total leukocyte (i.e., CD45+ cells) and myeloid cell infiltrates (p=0.045 and 0.049, respectively), but not of lymphoid cells infiltrates (Figure 10).

Regarding specific myeloid subpopulations, tumors from exercise mice showed a higher number of M2-like tumor-associated macrophages (p=0.028) and DCs (p=0.049) than the control group, and a trend toward a higher number of myeloid and plasmacytoid DCs (p=0.077 for both subsets) was also found (Figure 11). On the other hand, no significant between-group differences were found for the different lymphoid subsets (Figure 12).

Serum cytokines/chemokines

No significant group by time interaction effect was found for the levels of circulating cytokines/chemokines we studied (Table 4).

Table 4. Serum cytokines/chemokines by group.

Cytokines/ chemokines	Group	N	Baseline	Postintervention	Group (p)	Time (p)	Group by time (p)
IL6 (pg/mL)	Control	10	45 ± 8	63 ± 12	0.748	0.819	0.172
	Exercise	8	73 ± 35	48 ± 15			
IL15 (pg/mL)	Control	5	599 ± 313	282 ± 81	0.526	0.351	0.453
	Exercise	5	321 ± 203	285 ± 64			
MCP1 (pg/mL)	Control	5	183 ± 44	172 ± 77	0.300	0.898	0.878
	Exercise	3	87 ± 6	85 ± 31			
TNFα (pg/mL)	Control	4	10 ± 2	7 ± 3	0.753	0.619	0.545
	Exercise	3	10 ± 1	10 ± 6			
VEGF (pg/mL)	Control	9	0.9 ± 0.2	2.8 ± 1.7	0.220	0.712	0.260
	Exercise	5	6.3 ± 5.0	2.6 ± 1.6			

Data are mean ± SEM. Abbreviations: IL, interleukin; MCP, monocyte chemoattractant protein-1; TNFα, tumor necrosis factor α; and VEGF, vascular endothelial growth factor.

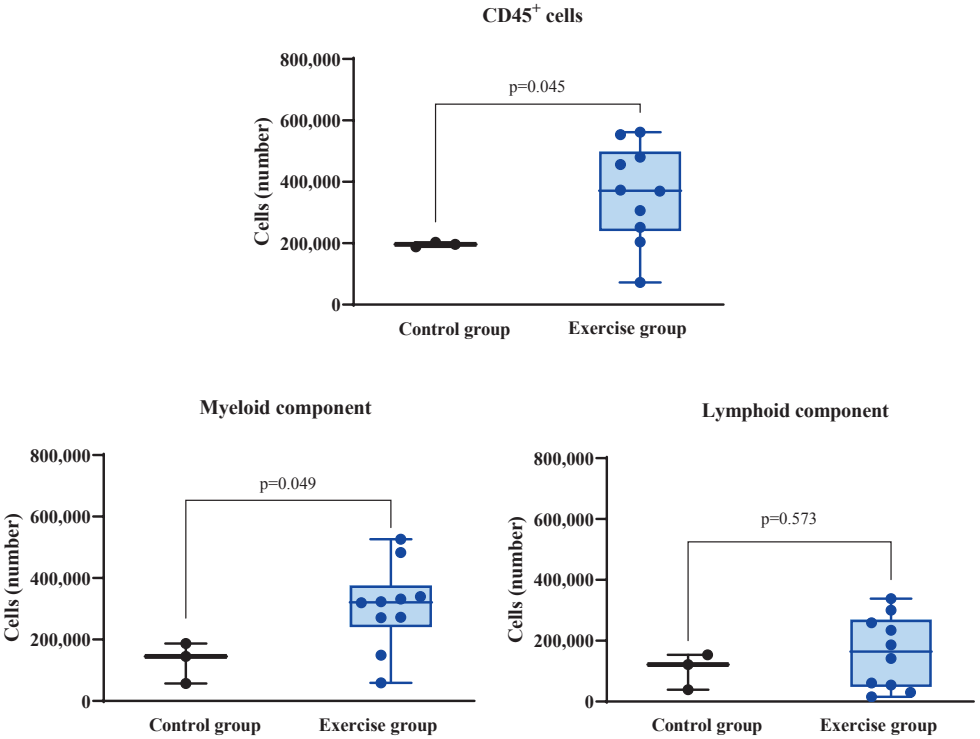


Figure 10. Total leukocyte (i.e., all CD45+ cells), myeloid (macrophages and myeloid-derived suppressor cells [CD11B+], and dendritic cells [CD11C+]), and lymphoid (T [CD3+] and B lymphocytes [B220+], and NK cells [NK1.1+]) infiltrates in 'hot' tumors by group. Data are mean ± SEM, with individual data points and p-values for the between group comparison also shown. N with data = 3 and 10 (control and exercise group, respectively).

Table 3. Immune cell phenotyping in peripheral blood by group.
Data are mean (%) ± SEM. Total N with complete data = 6 and 10 for the control and exercise group, respectively. Abbreviations: DCs, dendritic cells; MDCS, myeloid-derived suppressor cells.

type (CD markers)	Groups	Baseline	Postintervention	Group (p)	Time (p)	Group by time (p)
	Control	98.5 ± 0.2	85.7 ± 13.3	0.696	0.078	0.707
	Exercise	98.4 ± 0.3	79.1 ± 10.3			
	Control	31.3 ± 2.1	10.0 ± 1.9	0.324	<0.001	0.826
)	Exercise	35.7 ± 2.8	13.3 ± 3.5			
s	Control	31.5 ± 1.7	3.5 ± 1.2	0.850	<0.001	0.472
)	Exercise	30.1 ± 1.1	4.3 ± 1.6			
cells	Control	0.3 ± 0.1	0.1 ± 0.1	0.346	0.002	0.432
)	Exercise	0.4 ± 0.1	0.2 ± 0.1			
1B cells	Control	27.2 ± 3.6	83.6 ± 3.5	0.372	<0.001	0.536
)	Exercise	25.1 ± 2.3	77.6 ± 4.4			
1C cells	Control	1.2 ± 0.1	0.6 ± 0.2	0.520	0.416	0.163
)	Exercise	1.0 ± 0.2	1.2 ± 0.3			
ll subsets						
	Control	72.3 ± 1.6	61.6 ± 1.9	0.928	<0.001	0.756
CD4)	Exercise	72.0 ± 1.1	62.4 ± 3.0			
	Control	18.9 ± 0.2	28.2 ± 3.0	0.867	0.001	0.645
CD8)	Exercise	19.4 ± 0.5	27.1 ± 2.0			
ifferentiation						
	Control	0.7 ± 0.2	6.5 ± 2.8	0.753	0.002	0.876
CD8 CD62L CD44)	Exercise	1.0 ± 0.2	7.3 ± 2.0			
fferentiated effector memory T cells	Control	87.1 ± 1.1	74.2 ± 3.3	0.120	<0.001	0.514
CD8 CD62L CD44)	Exercise	82.9 ± 2.6	67.2 ± 2.8			
ory T cells	Control	12.1 ± 1.0	19.3 ± 2.6	0.247	0.012	0.833
CD8 CD62L CD44)	Exercise	15.9 ± 2.6	24.3 ± 2.8			
ry T cells	Control	0.6 ± 0.2	1.5 ± 0.8	0.393	0.021	0.453
CD8 CD62L CD44)	Exercise	0.6 ± 0.1	2.2 ± 0.5			
ecent activation						
	Control	0.1 ± 0.0	0.4 ± 0.3	0.729	0.090	0.543
CD8 OX40)	Exercise	0.1 ± 0.0	0.6 ± 0.3			
	Control	0.8 ± 0.5	0.2 ± 0.1	0.500	0.117	0.304
CD8 41BB+)	Exercise	0.4 ± 0.2	0.3 ± 0.1			
ustained activation						
	Control	0.4 ± 0.2	0.2 ± 0.1	0.174	0.004	0.187
CD8 LAG3)	Exercise	0.2 ± 0.0	0.1 ± 0.0			
	Control	0.6 ± 0.2	0.4 ± 0.1	0.572	0.138	0.835
CD8 TIM3)	Exercise	0.6 ± 0.2	0.2 ± 0.1			
	Control	3.4 ± 0.3	4.0 ± 0.2	0.217	0.307	0.755
CD8 PD1)	Exercise	4.1 ± 0.5	4.5 ± 0.5			
ifferentiation						
	Control	2.3 ± 0.6	1.8 ± 0.8	0.145	0.157	0.050
CD4 CD62L CD44)	Exercise	2.5 ± 0.5	5.1 ± 1.3			
fferentiated effector memory T cells	Control	76.0 ± 2.3	75.9 ± 4.7	0.506	0.104	0.106
CD4 CD62L CD44)	Exercise	78.1 ± 1.2	68.1 ± 4.4			
ory T cells	Control	18.7 ± 1.2	20.5 ± 3.7	0.689	0.113	0.323
CD4+ CD62L CD44)	Exercise	17.1 ± 1.2	24.6 ± 3.9			
ry T cells	Control	3.9 ± 0.7	2.1 ± 0.7	0.998	0.155	0.072
CD4 CD62L CD44)	Exercise	2.9 ± 0.5	3.2 ± 0.7			
ecent activation						
	Control	0.1 ± 0.0	0.0 ± 0.0	0.119	0.147	0.093
CD4 OX40)	Exercise	0.0 ± 0.0	0.6 ± 0.3			
	Control	0.7 ± 0.3	0.2 ± 0.1	0.811	0.236	0.220
CD4 41BB)	Exercise	0.4 ± 0.2	0.4 ± 0.2			
sustained activation						
	Control	0.4 ± 0.1	0.3 ± 0.2	0.263	0.570	0.570
CD4 LAG3)	Exercise	0.2 ± 0.1	0.2 ± 0.1			
	Control	0.7 ± 0.3	0.2 ± 0.1	0.294	0.044	0.475
CD4 TIM3)	Exercise	0.5 ± 0.2	0.2 ± 0.1			
	Control	7.5 ± 0.8	3.0 ± 0.7	0.074	0.001	0.442
CD4 PD1)	Exercise	5.9 ± 0.8	2.7 ± 0.4			
DSC	Control	25.3 ± 3.3	12.6 ± 1.2	0.193	<0.001	0.742
B Ly6G Ly6C^{high})	Exercise	22.5 ± 1.4	11.2 ± 1.2			
MDSC	Control	51.9 ± 5.6	75.6 ± 5.0	0.313	<0.001	0.722
B Ly6G Ly6C^{low})	Exercise	57.1 ± 2.1	77.3 ± 4.2			
	Control	22.8 ± 2.8	11.7 ± 4.1	0.632	0.009	0.752
B Ly6G Ly6C)	Exercise	20.3 ± 1.9	11.3 ± 3.6			
ophages	Control	99.5 ± 0.1	99.7 ± 0.2			

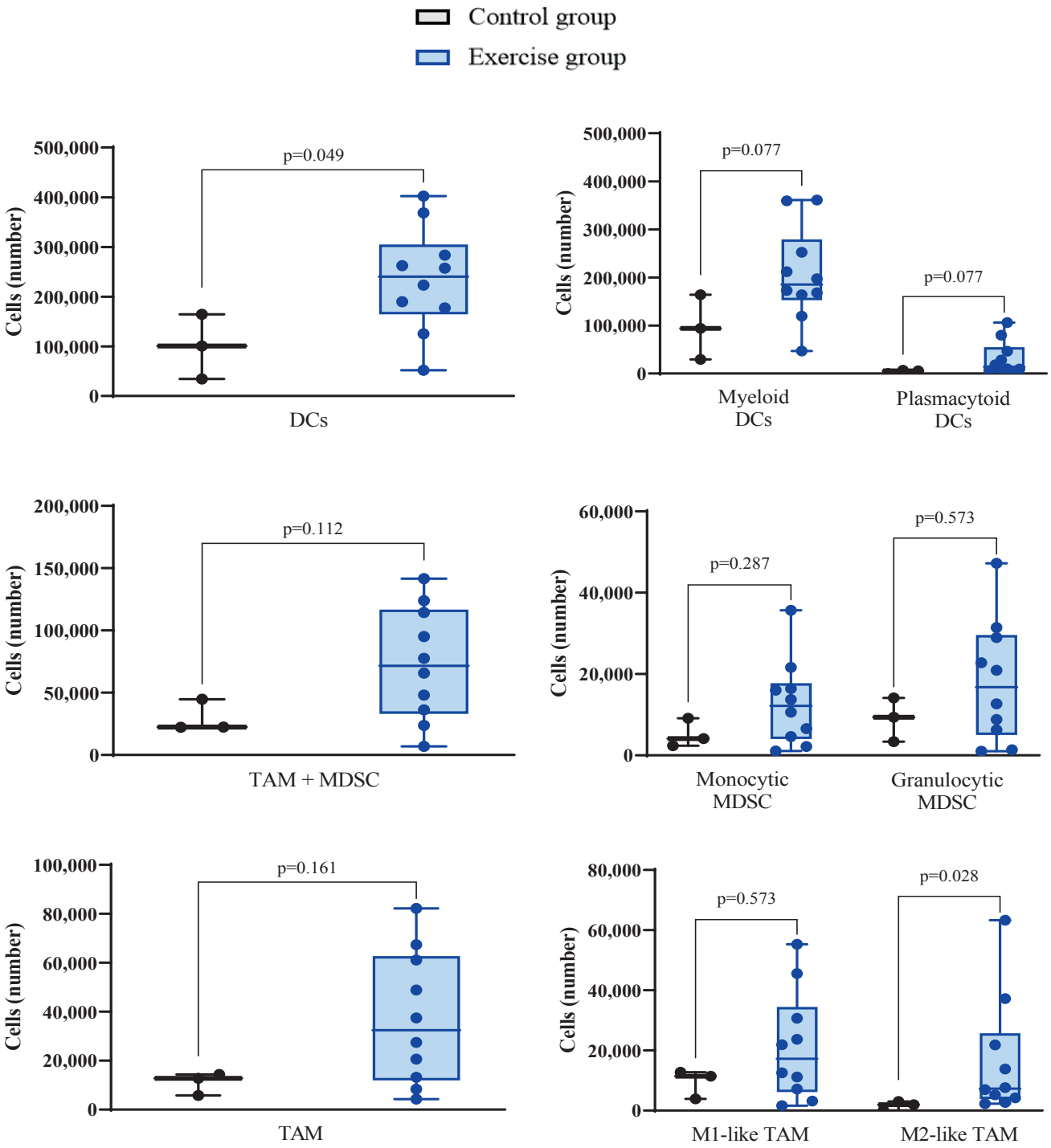


Figure 11. Specific myeloid subpopulation infiltrates in 'hot' tumors by study group. Data are mean ± SEM, with individual data points and p-values for between group comparison also shown. N with data = 3 and 10 (control and exercise group, respectively). Abbreviations: DCs, dendritic cells; MDSC, myeloid-derived suppressor cells; TAM, tumor associated macrophages.

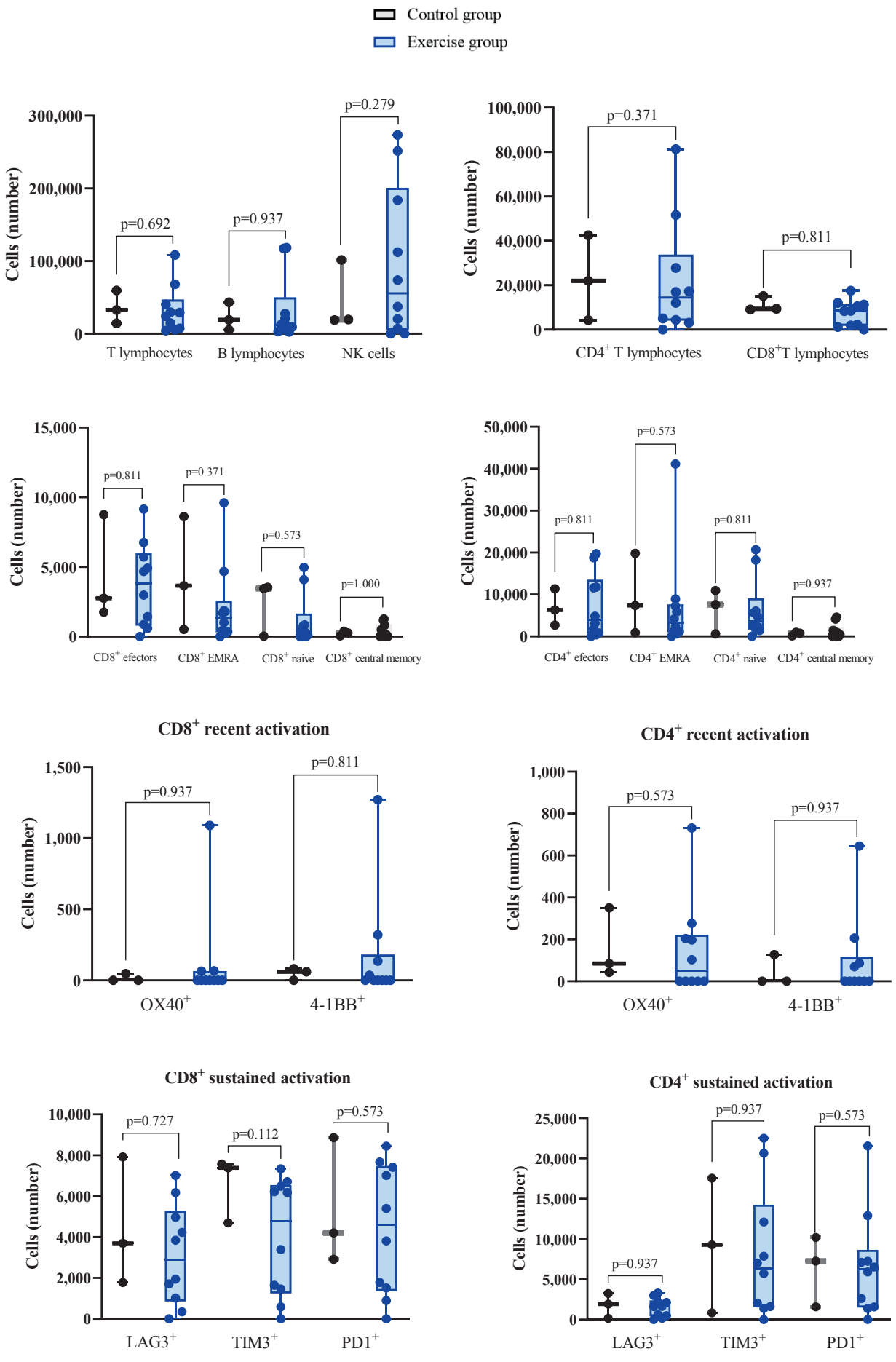


Figure 12. Specific lymphoid supopulation infiltrates in 'hot' tumors by study group. Data are mean ± SEM, with individual data points and p-values for between group comparison also shown. N with data = 3 and 10 (control and exercise group, respectively). Abbreviations: EMRA, terminally differentiated effector memory; NK, natutal killer cells.

DISCUSSION

The purpose of this preclinical study was to analyze the effects of a 5-week combined (aerobic and resistance) exercise intervention in the context of a highly aggressive tumor (one of the most treatment-resistant and life-threatening malignancies in children), HR-NB. Of note, because tumors were already palpable ~1 week before baseline measurements, we consider our model to allow studying exercise effects per se (without other concomitant anticancer interventions) after tumor onset (i.e., during the treatment phase) rather than in the prevention setting. Our main finding was that, despite the invasive nature of this tumor (i.e., inducing metastases in ~15% of all the tissues we studied and with a high proliferation rate, as reported by Ki67 results), this type of intervention attenuated the CRF decline associated with this malignancy, which at the muscle molecular level was supported by the finding of higher levels of CS and of several mitochondrial respiratory complexes (together with an improved antioxidant defense) in the exercise group compared with control mice. These exercise-induced benefits were accompanied by significant effects at the tumor tissue level (i.e., higher values of markers of apoptosis [caspase-3] and angiogenesis [VEGFR2] in the exercise group). The program also had an effect on immune cells, at least at the tumor level, with a trend towards a greater proportion of 'hot-like' (at least based on flow-cytometry analyses) tumors in the exercise group, together with higher (almost by more than twofold) total levels of tumor infiltrating leukocytes in the 'hot' tumors of this group. The latter in turn was partly accounted for by a higher infiltrate of myeloid cells. By contrast, as opposed to previous research in adult preclinical models (see discussion below) no differences were found for lymphoid cell infiltrates in the 'hot' tumors. On the other hand, no differences were found for other variables such as cancer progression (tumor volume and weight, metastasis, and tumor microenvironment), clinical severity, or survival, with these results overall expected in the context of an aggressive malignancy.

The exercise-induced benefits on CRF – and on some related variables such as mitochondrial oxidative capacity – in our preclinical model of HR-NB are of potential relevance when extrapolated to patients. In this effect, the benefits of physical exercise on physical function (and particularly CRF) in patients with cancer, particularly adults, are well documented [48,49], and indeed current guidelines recommend that these individuals engage in regular exercise in light of the aforementioned benefits, with subsequent improvements in fatigue and HRQoL [15]. CRF can be impaired in the context of cancer due to different mechanisms, including alterations in cardiac (e.g., cardiotoxicity), pulmonary, or muscle mitochondrial function associated with treatments, all of which are usually aggravated by the low physical activity levels of patients [50]. These adverse effects are not only found in adults, but also in pediatric patients, who typically present with an impaired physical capacity compared with their healthy peers (e.g., Z-score for CRF of -2) [38]. Our finding of an exercise-induced attenuation in CRF decline in the context of HR-NB is of clinical relevance also when considering that CRF levels are inversely associated with the risk of cancer-specific mortality in a dose-response manner [51], and lower levels of CRF have been associated with increased levels of fatigue specifically in children with cancer [38].

Although aerobic training is the exercise modality that has received the greatest attention in the cancer continuum, growing evidence supports the beneficial effects of resistance training, being associated with improvements in muscle strength, body composition (i.e., increased [or at least preserved] muscle mass), and reduced cancer-specific mortality [10,16,52,53]. Muscle strengthening activities represent indeed one of the core recommendations of international guidelines for physical activity [9]. The benefits of strength exercise would be particularly beneficial for patients with HR-NB, as reduced muscle mass has been identified as a predictor of poor prognosis in these individuals [54]. Compared with aerobic exercise, which has been extensively applied in rodents using forced (treadmill running or swimming) or voluntary (wheel running) exercise [12], one of the biggest challenges when implementing a strength training intervention in murine models of disease, particularly cancer, is to quantify and apply optimal training loads such as to induce significant gains in muscle mass and/or function. In this effect, the rapidly increasing tumor volume observed in the HR-NB model used in our study, as well as its location in the abdominal area, made it difficult to choose the right modality and intensity of strength exercise during the intervention. Potentially due to the aforementioned difficulties, no significant intervention effects were found for muscle strength or anabolic status indicators (pP70S6K/ P70S6K).

Physical exercise positively impacts the immune system of healthy people in general [55] with the evidence particularly strong (but not only) for a potential beneficial effect on NK lymphocytes [56-62], which participate in first line innate immune defense through their cytotoxic activity and release of effector cytokines [63]. In fact, a single exercise session suffices to mobilize NK lymphocytes into the bloodstream, although this might not necessarily translate into higher tumor (e.g., prostate) NK infiltrates [60,61]. Interestingly, a recent randomized controlled trial showed in per-protocol analyses higher NK cell infiltrates in tumor infiltration following 8 weeks of intense aerobic training for patients with prostate cancer [64]. However, current meta-analytical evidence does not support a significant long-term effect of exercise training intervention on the circulating levels of these cells or on their 'static response' (as assessed in vitro) in cancer patients/survivors [65]. Exercise can also stimulate other immune effectors against tumors beyond NK cells, such as CD8+ ('cytotoxic') T cells – which play a crucial role in the control of tumor development [66-69]. Thus, rodent forced running can enhance antitumor immune efficacy by increasing the intratumoral (breast cancer) ratio of CD8+ to CD4+ FoxP3+ T cells (also termed 'regulatory' or 'Treg', an immune subtype able to induce immune tolerance to tumors) [66]; or restrict tumor growth in multiple murine models of pancreatic ductal adenocarcinoma (PDA) through an expansion of lymphocyte clusters, particularly CD8+ T cells responsive to IL15 signaling (with these cells responsible for the observed reductions in tumor growth) [69]. Of note, patients with PDA who participated in a pre-operative exercise training program showed a significantly higher number of tumor-infiltrating CD8+ T lymphocytes compared with matched historical controls, and higher levels of intratumoral CD8+ T cells in the exercise arm were positively associated with survival [69]. However, other authors have reported no changes with exercise in the tumor infiltrates of mice that were subcutaneously inoculated with PDA cells [70].

One question that has not been addressed in the context of NB is the profile of immune cells infiltrating tumors after exercise training. In this effect, a novel result of our study was that the proportion of 'hot' tumors tended to be higher in the exercise group compared to control mice. Furthermore, these tumors showed a higher total immune (leukocyte) and myeloid infiltration compared to control animals. We believe this is a tantalizing finding when considering that the tumor we studied, HR-NB, the most aggressive NB type associated with recurrent somatic mutations and with *MYCN* oncogene amplification, can repress some antigens expressed in tumor cells (such as MCP-1/CCL2, required for chemoattraction of NK cells) and thus impair immune surveillance and immune cell recruitment inside tumors [71]. Indeed, HR-NB with *MYCN* amplification is often characterized by a sparse and limited immune infiltrate in the tumor microenvironment together with lower IFN pathway activation and chemokine expression, and infiltrating immune cells lack activation markers [72-74]. As such, the HR-NB tumor environment is often referred to as 'cold' or 'immune-deserted' [72]. NB tumors display low immunogenicity due to their low mutational load and lack of major histocompatibility complex class I (MHC-I) expression, with tumor infiltration by T and NK cells especially low in HR-NB and prognostic for survival [75]. Moreover, NB tumors employ a variety of immune evasion strategies that reduce infiltration and reactivity of immune cells (including DCs, as explained below), such as expression of immune checkpoint molecules, induction of immunosuppressive myeloid and stromal cells, as well as secretion of immunomodulatory mediators [75].

The higher total immune (leukocyte) and myeloid infiltration in the tumors of the exercise group compared to control animals was partly accounted for by a higher number of a myeloid subset, DCs. This result is potentially relevant result since the presence of tumor-infiltrating DCs is a favorable prognostic immune signature of NB tumors in general, independent from other variables used to stage and stratify treatment of patients such as *MYCN* amplification status or age at diagnosis [76]. Furthermore, NB tumors are able to induce DC dysregulation at multiple levels by inhibiting the maturation and function of these cells [77]. For instance, NB can induce DC dysfunction through inhibition of generation/differentiation of functionally active DCs by NB-derived gangliosides [78] (i.e., glycosphingolipids present on the surface of cells that are implicated in cancer development and progression, including tumor proliferation, invasion, angiogenesis, and metastasis [79]) or decreases in CD40 signaling (a stimulator of DC maturation and antitumor activity) [80] or other soluble factors [81]. In turn, DCs play an important role in anti-tumor immunity – notably in the context of NB – as they act as 'professional antigen-presenting cells' involved in initiating and coordinating the immune response against cancer, activating both adaptive (T cell-mediated) and innate (NK cell-mediated) arms of the cellular immune response cascade [82]. Within several tumors, myeloid DCs stimulate and expand tumor-specific effector T cells through IL12 signaling, with a high number of this type of DCs detected in spontaneous regressing tumor models – which suggests they might play a critical role for robust tumor control [83,84]. In turn, plasmacytoid DCs can activate NK cells and are efficient at killing HR-NB [85], which supports the use of immunotherapy with these cells as a potential strategy to decrease the risk of relapse in patients with HR-NB [86]. DCs represent indeed a potential immunotherapy to promote NB tumor regression, where there are some vaccines involving specific types of the cells that can be used to prevent this malignancy, as well as the combination of inflammatory factors and DCs as a substitute for chemotherapy [87].

In line with our results, Bianco et al. [88] found that an exercise (swimming) intervention increased intratumoral DC infiltrates in a mouse model of breast cancer compared to a non-trained group with the phenotype of these cells also different (i.e., higher CD80 and CD86 expression), suggesting that exercise training could influence the innate immune response to induce DC maturation and promote host antitumor immunity in the tumor microenvironment by presenting tumor antigens. Unfortunately, in our study we did not assess the maturity of the DCs. In any case, we found a trend towards a preferential mobilization of both plasmacytoid and myeloid DCs, with the latter being critical initiators of antitumor T cell responses [77]. On the other hand, the presence of immunosuppressive cells like total TAM, MDSCs, and T regulatory cells in tumors, for which we found no between-group differences in our study, correlate with poor clinical outcomes in NB [89,90].

While keeping in mind the limitation that we did not perform immunohistochemistry studies in TAM beyond flow cytometry analyses, an apparently unexpected, counterintuitive finding was the higher number of a specific subset of TAM, those with an M2-like phenotype, in the 'hot' tumors of exercised mice. Nonetheless, recent studies have reported overall comparable findings with regard to TAM in mouse models of breast (91) and lung cancer (92), where exercise in fact inhibited tumor growth, and this beneficial effect was independent of TAM infiltrates (92). Traditionally, macrophages are activated towards one of two polarization states, the classically activated M1 proinflammatory phenotype or the alternatively activated M2 immunosuppressive phenotype. Classically activated M1-like TAM induced by IFNγ and lipopolysaccharides are considered anti-tumor macrophages due to the expression of inducible nitric oxide synthase and the secretion of cytotoxic reactive oxygen species and proinflammatory cytokines, whereas TAM activated by IL4, the so-called M2 TAM, might be considered protumorigenic due to the expression of growth-promoting, proangiogenic, and extracellular matrix remodeling signals via VEGF, among other factors [93]. Importantly, M2-like TAM are also associated with a nonimmune stimulatory phagocytosis of apoptotic cancer cells known as 'efferocytosis' [94]. Other 'professional' phagocytes (such as monocytes and DCs) and 'non-professional' phagocytes (such as epithelial cells) can also participate in efferocytosis [94]. In this effect, the increase in apoptosis of tumor cells induced by the exercise program (as reflected by the higher levels of caspase-3 in the tumors of exercised mice compared to control animals) might explain, at least partly, the greater infiltrates of M2-like TAM inside the 'hot' tumors of the intervention group compared to control mice, as well as the increase in myeloid cells in general and DCs in particular (i.e., theoretically to induce efferocytosis).

Our findings are overall in line with the growing evidence on the benefits of physical exercise for improving immune cell effectors [13], albeit different types of cells can be mobilized into the tumors depending on factors such as the exercise protocol (i.e., acute bout versus regular exercise training) or the type of tumor, with scarce evidence up to date translatable to pediatric solid tumors. For instance, a preclinical study reported

that physical exercise promoted the infiltration of immune cells (NK cells in particular) into several types of adult tumors by enhancing the expression of ligands for NK cell-activating receptors (e.g., NKG2D), which seemed dependent on acute exercise-induced release of epinephrine (which increased mobilization of these cells into the bloodstream) and IL6 (which ultimately increased NK cell infiltration into tumors) [95]. In the same line, exercise has been reported to improve the antitumoral activity of resident macrophages [12], reducing the tumor-induced accumulation of MDSC [96,97] or increasing neutrophil infiltrates into tumors [42]. There is also evidence that aerobic or resistance training during chemotherapy can attenuate the immunosuppressive effects of drugs in patients with breast cancer, at least on CD8+ cells [98], which correlates well with the aforementioned preclinical evidence that exercise can stimulate CD8+ cells to infiltrate tumors [66-69]. In any case, all of the aforementioned studies were conducted in adult tumors, which might explain, at least partly, the fact that we found no between-group differences in CD8+ lymphocyte infiltrates. On the other hand, the finding of a significantly higher proportion of circulating CD4 naïve T lymphocytes in our exercise group is overall in line with previous data in humans showing 'younger looking' T-cell profiles in physically trained compared to inactive individuals, with overall fewer T-cells exhibiting phenotypes associated with differentiation and exhaustion, and greater frequencies of naïve T-cells and or recent thymic emigrants that are capable of responding to novel antigens [99–101].

The exercise intervention had a significant effect on two important variables with a major influence in cancer prognosis in general, tumor angiogenesis and apoptosis, with changes in both outcomes potentially explaining some of the biological effects of exercise against cancer development [102]. We found a potential increase in tumor angiogenesis capacity (as reflected by a higher % of VEGFR2-positive vessels inside the tumors despite no differences in total vessel count) with exercise training, which correlates with the aforementioned result for M2-like TAM and the overall proangiogenic effect of these cells [93]. Although there is no unanimity on the effects of exercise on tumor angiogenesis in general [12], emerging data indicates that both acute and chronic (repeated) aerobic exercise stimulate favorable improvements in intratumoral perfusion/vascularization and hypoxia in orthotopic models of human breast cancer and murine prostate cancer [103–105]. Moreover, studies using mouse models of cancers primarily found in the adult population (melanoma, pancreatic, breast, and prostate cancer) have demonstrated that exercise can remodel tumor vasculature and improve chemotherapy efficacy by increasing drug delivery [103–108]. In fact, a preclinical study found that exercise remodeled the vasculature of Ewing sarcoma to reduce vessel hyperpermeability and hypoxia, potentially via modulation of endothelial cell sphingosine-1-phosphate receptors 1 and 2, thereby improving doxorubicin delivery to tumors [23]. Thus, exercise may promote a shift towards a more 'normalized' tumor microenvironment (possibly via upregulation of regional and local physiologic angiogenesis) [109].

Controversy exists on the potential direct antitumoral effects of physical exercise in adult cancers. A potential mechanism might be the release of myokines from muscles associated with exercise or 'exerkines' (i.e., 'signalling molecules released from different tissues in response to acute and/or chronic exercise, which exert their effects through endocrine, paracrine and/or autocrine pathways' [110] in general). In this effect, we found no changes associated with the intervention in the blood levels of several exerkines (IL6, IL15, or VEGF), or of cytokines/chemokines associated with cancer development (MCP1 [111]) or cancer-related inflammation (TNFα [112]). There is some evidence that 'exercise-conditioned' serum might reduce tumor growth in vitro [113] (e.g., prostate cancer [114–116]), as first demonstrated by Rundqvist et al. [117]. However, other studies have failed to find such benefits after exercise training, even in the case of serum obtained immediately after an acute exercise bout [118,119]. There is also mixed evidence as to whether exercise training could reduce tumor growth in vivo, as reflected by two recent meta-analyses with conflicting results on the effects of physical exercise on tumor growth and metastasis in preclinical models [11,120]. In accordance with the present study, in which no exercise benefits were found on tumor weight or metastasis, other studies have also failed to find benefitial effects of physical exercise on tumor progression. Woods et al. [121] reported no significant differences in breast cancer tumor growth among mice randomized to 14 days of treadmill running compared with sedentary controls. Jones et al. [103] found that breast tumors grew at comparable rates in exercising and sedentary animals. The same group also found that exercise did not inhibit primary cancer progression in an orthotopic model of murine prostate cancer, although it did favorably alter genes responsible for metastatic dissemination in the primary tumor with a shift toward a suppression of distant metastases [104]. On the other hand, other preclinical studies have found a decrease in tumor growth with exercise training [66–69,95,102,122–124], and therefore further research is needed to confirm our findings, as well as to elucidate whether the tumor itself (e.g., not only type, but also method and timing of implantation), the exercise intervention (e.g., timing of exercise initiation, exercise type or intensity) or animal characteristics (e.g., immune deficient versus immune competent versus transgenic) could condition these effects. Notably, studies with the same tumor (B16-F10 melanoma) have reported increased or unchanged intratumoral NK infiltrates if the exercise intervention started before [95] or after [125] implantation, respectively, thereby suggesting that a 'pre-conditioning effect' prior to tumor onset may be needed to enhance NK cell infiltration. There is also high variability in the exercise training dose across preclinical studies, and although it is assumed that the same treadmill velocity would fit all mouse strains and experimental conditions, the relative exercise intensity for instance differs between C57BL/6 and Friend Virus B (FVB) female mice (67). This may explain, at least in partly, why preclinical studies can show different results even with similar exercise protocols (7,52,56). On the other hand, the epidemiological evidence for human cancers refers mainly to regular physical activity (e.g., walking or brisk walking) whereas supervised regular exercise (defined as a 'subset of physical activity that is planned, structured and repetitive and has the objective of improving or maintaining physical fitness'), which was the model used in our study (i.e., mouse forced treadmill running) is a proxy but not a perfect surrogate for physical activity and is thought to induce more profound molecular adaptations than physical activity [12]. In fact, the exercise doses used in preclinical studies might exceed human behavior [67].

No between-group differences were found for the gene expression associated with the tumor microenvironment, with a quasi-significant trend towards higher values of *Tgfb1* transcript levels in the intervention group (p=0.067). TGF-β is a member of a multifunctional cytokine family that is involved in essentially all aspects on cancer. On the one hand, TGF-β regulates cell proliferation [126]. TGF-β1, the most common isoform in human cancers, inhibits proliferation and induces apoptosis in various normal and premalignant human epithelial cells and its essential signaling intermediates (TβRII and Smad4), are therefore considered tumor suppressors. The anti-oncogenic function of this pathway is supported by the frequent occurrence in cancer cells of genetic and epigenetic alterations that abolish its growth-inhibitory function [127]. Interestingly, TGF-β1 is also considered a myokine, although it primarily induces paracrine effects (that is, in proximity to muscle cells) [110]. On the other hand, however, all advanced human tumors overproduce TGF-β, whose autocrine and paracrine actions in most instances promote tumor growth, invasion, and metastasis [128]. TGF-β also suppresses proliferation and differentiation of lymphocytes, including cytolytic T cells, NK cells and macrophages, thus preventing effective eradication of the developing tumor by the host immune system [127].

Some limitations of the present study should be acknowledged, such as the small sample size available for several outcomes. Due to ethical reasons we could not study very young mice (i.e., aged <2-3 weeks), which would have mimicked the very early occurrence of NB in children's life, and we could not assess acute exercise effects at the blood or tumor level in order to avoid sacrificing an excessive number of animals. For similar ethical reasons, we did not perform additional blood sampling between baseline assessments and the end of the intervention (i.e., before the tumors had grown significantly) and thus we cannot discard that a certain immunosuppressive effect of increasing tumor burden may have attenuated potential exercise benefits. On the other hand, we did not assess the effects of the exercise intervention on immune cells in the absence of tumor burden (i.e., in healthy mice). In turn, a major strength is having analyzed exercise effects in a preclinical model of a pediatric cancer, for which scarce evidence exists compared with adult cancers. Indeed, we have used a very aggressive tumor model developed by our group for which there is only one previous published study [40], where it was shown that these tumors are responsive to mCelyvir (i.e., autologous mesenchymal cells that carry an oncolytic adenovirus inside and is considered a form of cancer immunotherapy). In addition, we assessed a comprehensive range of outcomes. Other strengths and novelties include the use of a flow diagram (which precludes showing data from 'convenient samples') and resistance training in mice, and particularly, the tumor model we used, which allows to recapitulate the main features shown in patients. Indeed, we used an induced HR-NB model in which tumor cells are orthotopically implanted in the suprarenal area of 129/SvJ wild type mice [128,129]. Thus, tumor develop in a fully immunocompetent host, with the appropriate tissue microenvironment, mimicking the conditions that allow the interaction of the immune system with the developing tumor [130].

In summary, although no benefits were observed in tumor progression, clinical severity or survival rates, the present study support the effectiveness of a combined exercise intervention (aerobic + strength) for attenuating physical function decline in a mouse model of HR-NB, also exerting some immune benefits within the tumor (see Graphical Abstract for a summary of the main study findings). Thus, these findings shed new insights into the potential role of exercise as a potential co-adjuvant therapy in pediatric patients with solid tumors, as well as of potential differences in immune responses compared to adult malignancies.

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Supplemental file 1. Primary and secondary antibodies used in this study for western blot analyses of markers of muscle molecular adaptations to training at the skeletal muscle level.

	Antibody	Dilution	Provider	Reference
Primary antibodies	Catalase	1:1000	Sigma	C0979
	Citrate synthase	1:1000	Abcam	ab96600
	Gluthathione reductase	1:1000	Abcam	ab16801
	mtSOD	1:1000	MERCK Millipore	06-984
	OXPHOS rodent WB antibody cocktail*	1:250	Abcam	ab110413
	P70S6K	1:1000	Cell Signaling	9202
	pP70S6K (Thr389)	1:1000	Cell Signaling	9205
Secondary antibodies	Goat Anti-Mouse	1:3000	Abcam	ab205719
	Goat Anti-Rabbit	1:3000	Abcam	ab205718

Abbreviations: mtSOD, mitochondrial superoxide dismutase; OXPHOS, oxidative phosphorylation system; P70S6K, ribosomal protein p70 S6 kinase; pP70S6K, activated/phosphorylated P70S6K; WB, western blotting. Symbol: *This cocktail allows to determine all five mitochondrial respiratory complexes (CI to CV) simultaneously.

Supplemental file 2. Primary and secondary antibodies used for immunohistochemical analyses in tumors.

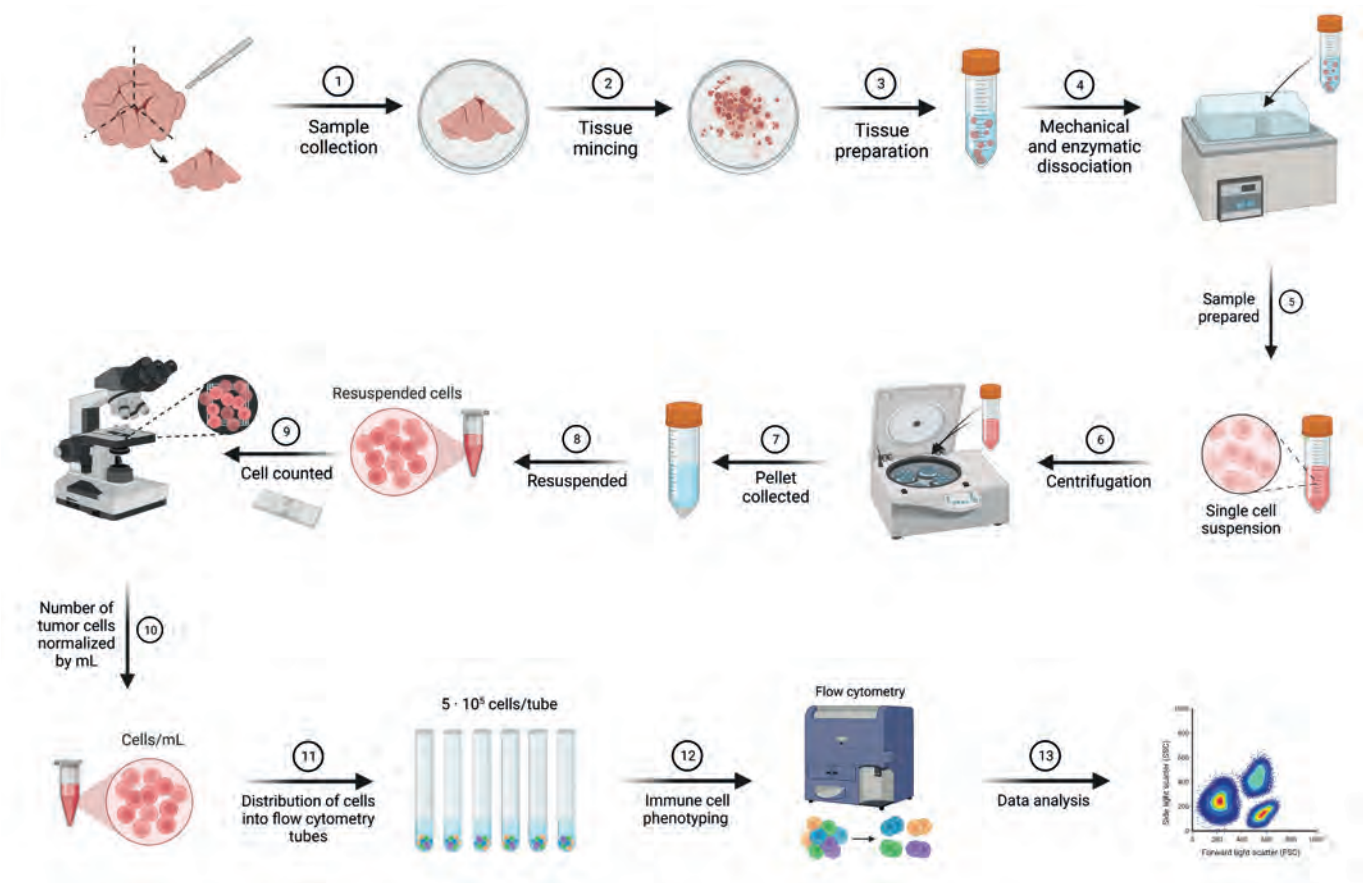
Antibody	Dilution	Provider	Reference
Cleaved Caspase-3 (Asp175) (5A1E)	1:400	Cell Signaling	9664
Histone H3 (D1H2)	1:100	Cell Signaling	4499
Ki-67 (D3B5)	1:1000	Cell Signaling	12202
VEGFR2 (55B11)	1:500	Cell Signaling	2479
Von Willebrand Factor (Factor VIII-related antigen)	1:100	Agilent	GA527

Abbreviation: VEGFR2, vascular endothelial growth factor receptor 2.

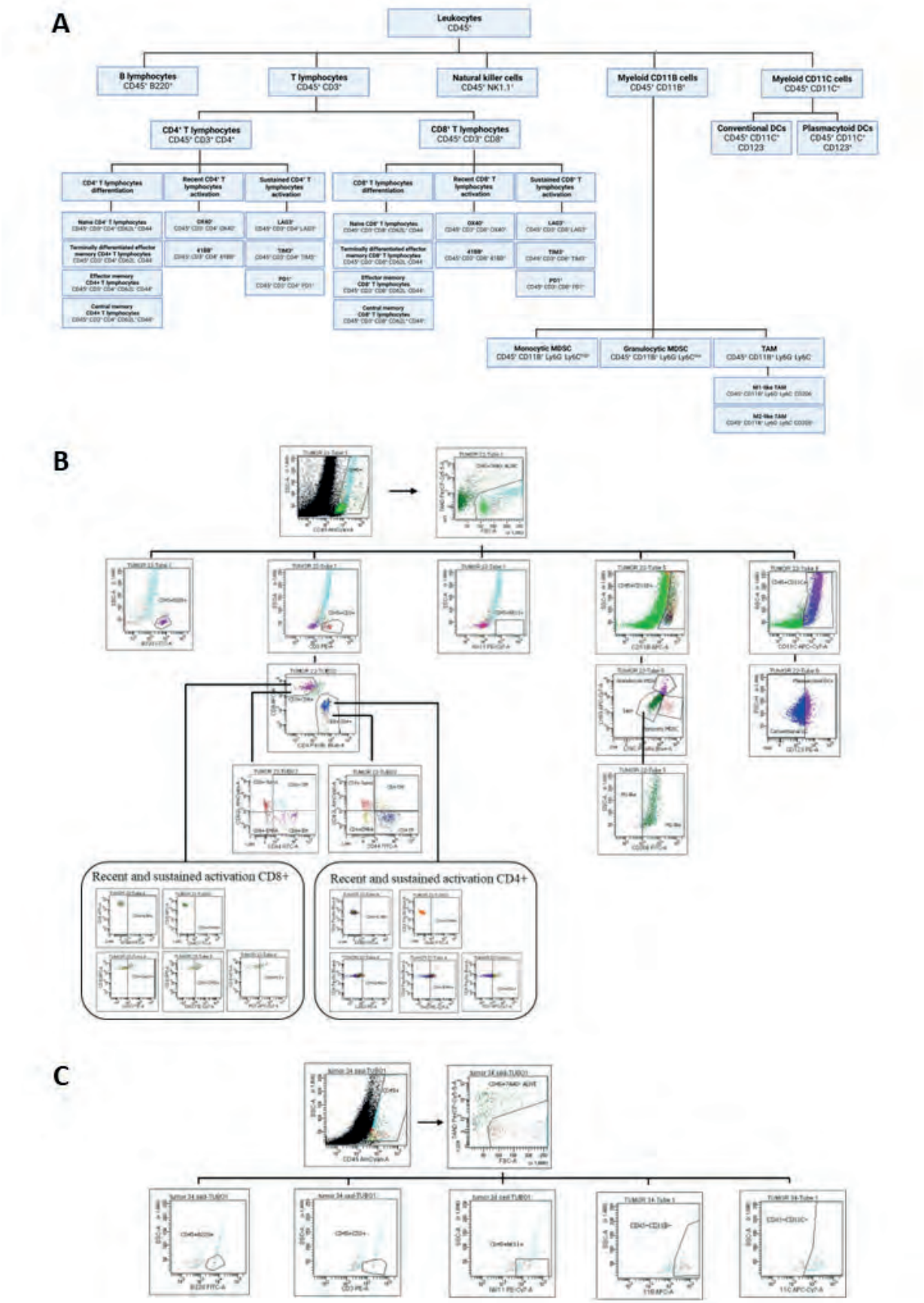
Supplemental file 3. Flow cytometry antibodies.

Tube	Antibody	Fluorophore	Provider	Reference
1	CD11b	APC	BioLegend	101212
	CD11c	APC/Cyanine7	BioLegend	117324
	CD45	Brilliant Violet 510™	BioLegend	103138
	CD45R/B220	FITC	BioLegend	103206
	CD3ε	PE	BioLegend	100308
	NK-1.1	PE/Cy7	BioLegend	108714
	7AAD Viability Staining	PERCP/CY7	BioLegend	420404
2	CD8a	APC	BioLegend	100712
	Ly-6A/E (Sca-1)	APC/Cyanine7	BioLegend	108125
	CD62L	Brilliant Violet 510™	BioLegend	104441
	CD4	Pacific Blue™	BioLegend	100531
	CD44	FITC	BioLegend	103022
	CD122 (IL-2Rβ)	PE	BioLegend	123209
	CD95	PE/Cy7	BD	557653
	7AAD Viability Staining	PERCP/CY7	BioLegend	420404
3	CD8a	APC	BioLegend	100712
	CD25	APC/Cyanine7	BioLegend	102026
	CD45	Brilliant Violet 510™	BioLegend	103138
	CD134 / OX40	FITC	Fisher	MA5-17917
	CD4	Pacific Blue™	BioLegend	100531
	CD3ε	PE	BioLegend	100308
	CD152 (CTLA-4)	PE/Cy7	BioLegend	106313
	7AAD Viability Staining	PERCP/CY7	BioLegend	420404
4	CD137 (4-1BB)	FITC	BioLegend	558975
	CD223 (LAG-3)	PE	BioLegend	125207
	CD366 (Tim-3)	PE/Cy7	BioLegend	119715
	CD279 (PD-1)	APC/Cy7	BioLegend	135223
	7AAD Viability Staining	PERCP/CY7	BioLegend	420404
5	CD11b	APC	BioLegend	101212
	Ly-6G	APC/Cy7	BioLegend	127624
	CD45	Brilliant Violet 510™	BioLegend	103138
	CD206 (MMR)	FITC	BioLegend	141704
	Ly-6C	Pacific Blue™	BioLegend	128013
	7AAD Viability Staining	PERCP/CY7	BioLegend	420404
6	CD11b	APC	BioLegend	101212
	CD11c	APC/Cyanine7	BioLegend	117324
	CD45	Brilliant Violet 510™	BioLegend	103138
	CD103	FITC	BioLegend	121419
	CD123	PE	BioLegend	106005
	7AAD Viability Staining	PERCP/CY7	BioLegend	420404

Supplemental file 4. Procedures used from tumor sample collection to final immune cell phenotyping with flow cytometry.



Supplemental file 5. Immunophenotype determination in the tumors with flow cytometry. **A**. Strategy to analyze flow cytometry data. **B**. Flow cytometry plots from an example of 'hot-like' tumor. When the viability of CD45+ (total leukocytes) infiltrates in the tumor using 7AAD labelling was analyzed, it was found that 38.9% of these cells were alive. Thus, the main immune subsets were subsequently detectable. **C**. Flow cytometry plots from an example of 'cold-like' tumor. When the viability of CD45+ (total leukocytes) infiltrates in the tumor using 7AAD labelling was analyzed, it was found that 1.2% of these cells were alive. This value represented a 0.3% of the total 'events' analyzed. Thus, the main immune subsets were subsequently undetectable.



The anti-inflammatory and bioregulatory effects of habitual exercise in high-fat diet-induced obesity involve crown-like structures and MCP-1 in white adipose tissue.

Isabel Gálvez[1,2], María Dolores Hinchado[1,3*], Leticia Martín-Cordero[1,4], Francisco Javier Morán-Plata[3], Gerry Graham[5], Javier Francisco-Morcillo[6], Eduardo Ortega[1,3*]

[1] Immunophyisiology Research Group, Instituto Universitario de Investigación Biosanitaria de Extremadura (INUBE), 06071 Badajoz, Spain
[2] Immunophysiology Research Group, Nursing Department, Faculty of Medicine and Health Sciences, University of Extremadura, 06071 Badajoz, Spain.
[3] Immunophysiology Research Group, Physiology Department, Faculty of Sciences, University of Extremadura, 06071 Badajoz, Spain
[4] Immunophysiology Research Group, Nursing Department, University Center of Plasencia, University of Extremadura, 10600 Plasencia, Spain
[5] Chemokine Research Group; Institute of Infection, Immunity and Inflammation; College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8TT, UK
[6] Cell Biology Area; Anatomy, Cell Biology and Zoology Department, Faculty of Sciences, University of Extremadura, 06071 Badajoz, Spain

ABSTRACT

Macrophage accumulation in the adipose tissue and changes in their inflammatory phenotype is a hallmark of obesity-induced inflammation, notably forming inflammatory structures known as "crown-like structures (CLS)". Exercise can be a key strategy to improve inflammation-related complications, but it is crucial to consider that, although exercise generally exerts systemic and local anti-inflammatory effects, this depends on the basal inflammatory status and exercise modality. In this context, the "bioregulatory effect of exercise" implies to achieve the reduction or prevention of an excessive inflammatory response and also the preservation or stimulation of the innate response. In the present work, our aim was to evaluate the effect of regular exercise on adipose tissue inflammation in high-fat diet-induced obesity in mice, as reflected by macrophage infiltration and phenotype, and CLS formation, together with a potential role for the chemokine MCP-1 in this process. Results showed that obesity is associated with greater MCP-1 expression ($p<0.05$), macrophage accumulation ($p<0.05$), and CLS presence ($p<0.001$). Regular exercise reduced macrophage accumulation ($p<0.05$), MCP-1 expression ($p<0.01$), and CLS presence ($p<0.05$) in obese mice; while it increased macrophage and CLS presence ($p<0.01$), MCP-1 expression ($p<0.05$), and M2 polarization ($p<0.05$) in lean mice. MCP-1 was associated with the proliferation of CLS, showing the first image demonstrating a potential role of this chemokine in the development of these structures. Altogether, these results confirm, for the first time, the "bioregulatory effect of exercise" in the adipose tissue: reducing inflammation in individuals with an elevated inflammatory set-point, but stimulating this response of the immune system in healthy individuals.

Key words: Inflammation. Macrophages. CCL2. CLS. Phenotype.

INTRODUCTION

Obesity and its related comorbidities (insulin resistance, type 2 diabetes, atherosclerosis, cardiovascular disease, cancer) represent one of the greatest health burdens of our time. It is well-known that obesity induces a state of chronic low-grade inflammation, characterized by high circulating levels of inflammatory cytokines and other inflammatory mediators (10,19). These anomalies in the immune and inflammatory activities in obesity are also strongly associated to altered neuroendocrine responses, dysregulated feedback mechanisms between the immune and stress responses (32, 33, 40), and abnormal metabolic homeostasis, all of which can lead to a cluster of chronic metabolic disorders or metabolic syndrome (10, 19).

Obesity-induced low-grade inflammation is also present locally, particularly in the white adipose tissue (WAT), as characterized by the increased cytokine/chemokine/adipokine expression and immune cell activation and infiltration (8, 26). In fact, WAT is now recognized as the main inflammatory source that mediates obesity-induced inflammation and metabolic alterations (24) and has also been proposed to be the main site where low-grade systemic inflammation begins (53). Under metabolic stress, adipocytes produce inflammatory mediators and chemoattractant molecules that can both activate and recruit resident and non-resident immune cells, namely macrophages

*Correspondence to:
E. Ortega (orincon@unex.es), M.D. Hinchado (mhinsan@unex.es): Immunophysiology Research Group, Physiology Department, Faculty of Sciences; and Instituto Universitario de Investigación Biosanitaria de Extremadura (INUBE), University of Extremadura, 06071 Badajoz, Spain.

and T-lymphocytes, that perpetuate WAT inflammation (43). Macrophages are key mediators in obesity-induced low-grade inflammation, producing the majority of cytokines in the adipose tissue, particularly those responsible for the onset of insulin resistance, such as TNF-α (26, 36, 53, 57). In this context, macrophage accumulation in the WAT is recognized as a hallmark of obesity-induced inflammation (53). Although the initial inflammatory triggers in WAT are still almost unknown, it has been proposed that obesity-induced adipose tissue remodelling gives rise to signals (lipids accumulation, mechanical stress, adipocyte hyperplasia and hypertrophy and subsequent hypoxia and cell death) capable of initiating an inflammatory response by promoting macrophage recruitment and activation, which in turn promotes the progression of systemic insulin resistance (9, 24, 28, 36, 45, 48, 60). Moreover, several chemotactic factors have been implicated in the process of macrophage infiltration, with CCL2/MCP-1 playing a crucial but controversial role in the recruitment of inflammatory monocytes and macrophages into WAT (20, 21, 22, 29, 52, 58).

In this way, a notable feature of macrophage infiltration in the WAT of obese subjects is the characteristic formation of clusters of macrophages that surround hypertrophied, dead adipocytes. These unique morphological elements are known as "crown-like structures" (CLS) and represent a major characteristic of WAT inflammation (9, 36). In CLS, macrophages form syncytia that sequester and ingest adipocyte debris as an initially protective response that later becomes a persistent site of macrophage activation (9), with increased expression of TNF-α and IL-6 (50). In obesity, the vast majority of WAT macrophages are aggregated in CLS (9).

Macrophages change not only their number during obesity but also their location and inflammatory phenotype (28, 29, 53). They are typically divided into two groups based on whether they are classically (pro-inflammatory, M1) or alternatively activated (anti-inflammatory, M2). M1 macrophages are linked to cellular immunity and microbicidal activity as well as production of pro-inflammatory mediators that recruit other immune cells into the local inflammatory sites and polarize and activate other immune cells; whereas M2 macrophages are associated with production of anti-inflammatory mediators, tissue repair and remodeling processes, humoral immunity (8, 14, 26, 34), and maintenance of insulin sensitivity and glucose tolerance (37, 38). Hence, macrophages can play various roles in the initiation, progression, and resolution of inflammation (26). Obesity is associated with dramatic increases in M1 macrophages numbers, particularly in the WAT (14, 28) and specially in CLS (28, 29, 54). However, it is not clear yet how obesity increases this subpopulation: by a phenotypic switch of resident M2 macrophages to an M1 state, by recruitment and infiltration of pro-inflammatory monocytes (29), or by local proliferation (1, 59, 62). Conversely, it has also been shown that obesity induces the polarization of M2 macrophages (5, 61), which might suggest that the balance between these two subpopulations shapes obesity-induced inflammation (26).

Bearing all this in mind, exercise can be a key strategy to modulate obesity-induced low-grade inflammation, particularly WAT inflammation (7, 17, 51). The beneficial effects of moderate regular exercise are mediated through several anti-inflammatory mechanisms, such as the increased production and release of anti-inflammatory cytokines and the inhibition of the production of pro-inflammatory cytokines and the expression of co-stimulatory molecules (17). Another important mechanism can be the shift in WAT toward an anti-inflammatory phenotype with a reduction in macrophage infiltration, and an M1 phenotype switch (23). Nevertheless, the exact mechanisms by which exercise reduces WAT inflammation are still not completely understood (43). Moreover, although it is accepted that, in general, exercise exerts systemic and local anti-inflammatory effects (17), this depends on exercise modality and the basal inflammatory state or inflammatory set-point (15, 42), with a special focus on the presence of inflammatory pathologies in which basal inflammatory state is altered (11). Exercise-induced activation of the innate and/or inflammatory responses in healthy people reflects an immunophysiological adaptation in a situation of exercise-induced stress and vulnerability for the organism, being crucial for the defense against potential pathogenic attacks; while the anti-inflammatory effects induced by exercise in subjects with a pro-inflammatory set-point can be explained by the homeostatic adjustment preventing an excessive inflammatory response (42). In this context, whether this phenomenon also occurs in WAT is yet to be elucidated.

In addition, despite these advances, we have yet to elucidate many aspects of WAT macrophage function, infiltration, and polarization in lean and obese states, as well as the biology of cell interplay within CLS. Moreover, the means by which physiological and stress conditions influence adipose tissue immunity are largely unknown. It is important to note that most studies have been carried out in genetically obese animals, and that since CLS can be only determined by microscopical techniques, their complete characterization, including the effects of different anti-inflammatory strategies on these inflammatory elements, remains limited at present. In this work, we aim to study the effect of regular exercise on WAT inflammation in high-fat diet-induced obesity with a new approach to characterize macrophage infiltration, by using macrophage marker F4/80 (with the presence or absence of CD206 as inflammatory phenotype marker), and CLS formation; together with a potential role for the chemokine MCP-1 in this process. Moreover, once demonstrated if MCP-1 is involved in CLS formation and in the anti-inflammatory effect of regular exercise in the WAT of obese individuals, our second objective was to investigate whether these effects are the same or are different in the WAT of lean individuals, since they can be dependent on the basal set-point of inflammation, as proposed by the "bioregulatory effect of exercise" (42).

MATERIAL AND METHODS

Experimental Design

Thirty-four C57BL/6J mice (Envigo, Huntingdon, UK) were randomly allocated to one of two diets at 8 weeks of age, until sacrifice 18 weeks later. To obtain an experimental model of obesity, one group (n = 16) (obese group) was placed on a high-fat diet (HFD) (260HF diet; SAFE, Augy, France) containing 36% fat (58.8% of the energy from fat), which is optimal for the study of obesity and its complications in mice (15). The other group (n = 18) constituted the healthy control group (lean group) and was placed on standard laboratory rodent chow (SD) (A04 diet; SAFE, Augy, France), containing 3.1% fat (8.4% of the energy from fat). Each group was randomly divided into two sub-groups, sedentary and trained groups.

Mice had free access to food and water and were housed

individually, in a temperature- and humidity-controlled room (22 ± 1 °C; 60 ± 5%) with a 12 h light/12 h dark cycle (23:00–11:00 h light; 11:00–23:00 h dark). Olfactory and visual contact between mice was possible all along the study to avoid potential harmful effects of isolation.

After 10 weeks of the diet protocol, the group of obese trained mice (n = 8) and the group of lean trained mice (n = 8) were subjected to a protocol of habitual exercise for 8 weeks. After 12 h fasting and 72 h of rest for the trained groups, blood samples and visceral WAT were collected from anaesthetized animals (comparison obese vs. lean in sedentary and trained groups).

The study was approved by the Bioethics Committee for Animal Experimentation of the University of Extremadura (registry numbers 115/2015 for project DEP2015-66093-R; 70/2018 for project IB18011), in accordance with the National and European legislation for the protection of animals used for research.

Exercise Protocol

The protocol of regular exercise began at approximately 18 weeks of age, after 10 weeks of the diet protocol. Exercise training was carried out 3 days per week for 8 weeks, always at the same time in the active period (dark 11:00–23:00 h). Animals performed treadmill running (model 800, IITC Life Science Inc., Los Angeles, CA, USA) with no slope, with intensity and duration progression from 10 m/min for 10 min in the first week to 18 m/min for 45 min in the last week. A manual prodding technique instead of the traditional electrical shock was used to stimulate running, in order to avoid additional physiological stress. This protocol of moderate regular exercise is accepted to be able to induce physiological adaptations in mice (16, 44). Samples were obtained 72 h after the last training session to avoid the evaluation of the acute effects of exercise.

Body Measurements and Collection of Biological Samples

Body weight, nose-to-anus length, and food consumption measurements started the first week of the protocol and continued weekly for the entire lifespan of each mouse. Fasted animals were gas anaesthetized with isoflurane, by standard procedure (starting dose 3–5% isoflurane, maintenance dose 1.5–3% isoflurane). Biological samples were obtained from live, anaesthetized animals. Whole blood was drawn by cardiac puncture. Fasting blood glucose concentration and lipid profile including total cholesterol, high-density lipoprotein cholesterol (HDL-C), calculated low-density lipoprotein cholesterol (cLDL-C) and triglycerides (TG) were measured in whole blood (LUX®, Biochemical Systems International Srl, Arezzo, Italy) (15, 16, 31). Visceral WAT was carefully dissected via laparotomy. It was embedded in optimal cutting temperature (OCT) compound (Tissue-Tek®, Sakura Finetek Europe, Netherlands) and snap frozen in liquid nitrogen. Until further analysis, samples were stored at −80 °C.

Immunohistochemistry

WAT was cut into 14 µm sections in a cryostat (Microm, HM550, Thermo Fisher Scientific, Waltham, MA, USA) at −35 °C and mounted on Superfrost® Plus microscope slides (Thermo Fisher Scientific). Samples were stored at −20 °C until immunostaining procedure. A hydrophobic barrier pen (Vector Laboratories, Burlingame, CA, USA) was used to encircle the sections.

First, sections were fixed with 4% paraformaldehyde (PFA) in phosphate-buffer solution (PBS) (0.1 M, pH 7.4) for 5 minutes and then washed twice in 0.5% Triton® X-100 (Sigma-Aldrich, Saint Louis, MO, USA) in PBS solution for 10 minutes. A blocking buffer consisting of 10% bovine serum albumin (BSA, Sigma-Aldrich, Saint Louis, MO, USA) and 0.3% Triton® X-100 in PBS was applied for 1 hour in a humidified, light-protected chamber, at room temperature. Afterwards, one section was incubated with different combinations of conjugated antibodies against CD206 (CD206-Alexa Fluor® 594, BioLegend, San Diego, CA, USA; 1:250 dilution), F4/80 (F4/80-FITC, BioLegend, 1:250 dilution), and MCP-1 (CCL2/MCP-1-Alexa Fluor® 647, Novus Biologicals, Centennial, CO, USA, 1:250 dilution) diluted in antibody buffer (0.3% Triton® X-100, 1% BSA) and left overnight at 4 °C in the dark. Optimal concentrations of each antibody were determined after titration. Another section was incubated with the respective isotype control for each antibody, and a third section was a negative control, in order to ensure antibody specificity and lack of strong background signal.

The following day, samples were washed in PBS three times for 5 minutes and subsequently mounted in ProLong™ Gold Antifade Mountant with DAPI (4′,6-diamidino-2-phenylindole) (Invitrogen, Carlsbad, CA, USA). Slides were then stored at 4 °C in humidified, light-protected chambers until observation in the microscope.

Fluorescent images were acquired using a Zeiss Axio Imager M2 microscope at 40x magnification, with Zen software (Oberkochen, Germany). Representative snapshots were taken from each sample. For quantitative analysis, 10 random fields were photographed in each blinded sample. Macrophages were determined by F4/80+ cells, obtaining the mean number of macrophages per field. M2 macrophages were determined by CD206+ cells, obtaining the mean number of M2 macrophages per field. MCP-1 expression was assessed through mean fluorescent intensity (mfi) in 10 fields. Furthermore, samples were doubly stained with F4/80 and MCP-1 for morphological and immunohistochemical characterization. Mean number of crown-like structures, as determined by characteristic morphology and F4/80+ macrophage accumulation, was also counted in 10 random fields. Moreover, mean adipocyte size in 10 random fields (at least 5 adipocytes counted in each field) was measured in bright-field microscopy after standard haematoxylin and eosin staining in the frozen sections, using ImageJ software (Fiji).

Statistical Analysis

Values are expressed as mean ± standard error of the mean (SEM). The variables were normally distributed (tested by the Kolmogorov–Smirnov normality test). Statistical analyses were performed with GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA, USA). Student's t-test was used for comparisons between the pairs of groups (paired or non-paired samples). Two-way analysis of variance (ANOVA) test was used to evaluate the interaction between obesity and training on each parameter. Minimum significance level was set at $p < 0.05$.

RESULTS

Body and metabolic parameters

Table 1 shows the results regarding body and metabolic parameters in lean and obese mice, both sedentary and exercised. First, as expected, we confirmed that mice in the obese group presented higher fasting glucose concentrations and elevated levels of TG, total cholesterol, HDL-C, and cLDL-C. The protocol of habitual exercise decreased TG, and cLDL-C levels; and increased HDL-C levels in obese and lean animals, as previously shown in previous works in the same mice strain (16). Moreover, adipocyte size was greater in obese animals than in lean ones and after the exercise protocol, adipocyte size significantly decreased in both lean and obese mice. All of these responses confirm that our model of HFD-induced obesity and our model of exercise training are appropriate for evaluating physiological and metabolic effects in obese mice.

Table 1: Body weight and metabolic profile in obese and lean mice (sedentary and trained).

	Lean		Obese	
	Sedentary	Trained	Sedentary	Trained
Body weight (g)	30.2±1.3	24.9±1.1 **	42.6±1.5 •••	35.9±2.9 *
Adipocyte size (μm)	88.6±2.28	39.9±0.89 ***	118.2±3.16 •••	91.24±1.74 **
Fasting glucose (mg/dL)	203.5±14.5	196.4±25	286.7±21 ••	282.5±28
Total cholesterol	105.1±2.7	107±3	167.7±20 •••	178±25
HDL-C	45.2±2.3	54.1±3.5 *	58.2±5 •	79.5±1 **
cLDL-C	50.7±3.4	39.4±1.7 **	92.6±17 •	38.5±1*
TG (mg/dL)	86.6±2	77.1±0.5 ***	93.1±1.4 •	80.6±1 ***

Each value represents the mean ± SEM of the determinations (one per independent animal) in duplicate. * p<0.05, *** p<0.001 with respect to the corresponding sedentary group value; • p<0.05, •• p<0.01, ••• p<0.001 with respect to the lean sedentary group values. HDL-C high-density lipoprotein cholesterol; cLDL-C calculated low-density lipoprotein cholesterol; TG triglycerides.

Macrophage infiltration and their inflammatory phenotype in white adipose tissue

Both density of infiltrated macrophages and their inflammatory phenotype have been accepted as WAT inflammation biomarkers but have been scarcely evaluated directly in histological images in the context of obesity and exercise. We initially assessed the frequency and distribution of macrophages in the WAT in each group of animals, considering both representative histological images and more importantly, objective statistical data acquired from those images (Figure 1). As expected, number of F4/80+ cells (macrophages) were higher ($p<0.05$) in obese sedentary animals than in lean ones (Figure 1: graph E) as can be visually observed in the representative image A (lean) versus the representative image C in Figure 1. Obese mice that underwent the habitual exercise protocol presented a lower number of F4/80+ cells ($p<0.05$) than their sedentary controls (Figure 1: images C and D, graph E), while lean mice showed a higher number of F4/80+ cells ($p<0.01$) than in sedentary condition (Figure 1: images A and B, graph E). This differential behaviour in response to exercise in lean and obese mice was statistically significant ($p<0.01$) when compared by a two-way ANOVA test (Figure 1E). It is well known that macrophage infiltration in the adipose tissue in obese individuals is clearly related to increased body fat and adipocyte size (9, 36). In this context, although the lower macrophage infiltration in trained obese mice can be explained by the reduction in body weight and adipocyte size (Table 1), results clearly suggest that the influence of exercise on macrophage infiltration can be independent (or not only dependent) of fat mass, since in both groups body weight and adipocyte size decreased significantly and macrophage infiltration showed an opposite behaviour between both groups in response to exercise. In fact, adipocyte size in obese trained mice is still more than 100% higher than in lean trained mice (Table 1) and macrophage infiltration was significantly lower ($p<0.01$ in obese trained mice vs. lean trained mice; Student's t-test).

Results obtained regarding the inflammatory phenotype are shown in Figure 2. Number of cells expressing the CD206 marker (M2 macrophages) was similar in lean and obese sedentary mice (Figure 2: images A and C, graph E), but after the protocol of habitual exercise, the presence of these cells was increased only in lean mice (Figure 2: images A and B, graph E; $p<0.05$). Thus, the decrease in F4/80+ cells after exercise in obese animals (Figure 1) was not accompanied by a decrease in the M2 phenotype of macrophages. This effect of exercise was also significantly different in obese and lean mice (Figure 2E; $p<0.05$) when evaluated by a two-way ANOVA test.

MCP-1 expression in white adipose tissue

Figure 3 shows MCP-1 expression levels dispersed throughout the adipose tissue in the different groups. Expression levels (mfi) were higher in sedentary obese animals than in lean ones (Figure 3: images A and C, graph E; $p<0.05$); but in the obese mice, after the regular exercise protocol, this expression was lower than in sedentary condition (Figure 3: images C and D, graph E; $p<0.01$). However, MCP-1 expression levels increased in lean mice after the exercise protocol (Figure 3: images A and B, graph E; $p<0.05$). This differential effect of exercise in lean and obese mice was statistically significant (Figure 3E; $p<0.01$) when evaluated by a two-way ANOVA test, and reflected in general the same response to obesity and exercise than that observed in the number of infiltrated macrophages (Figure 1).

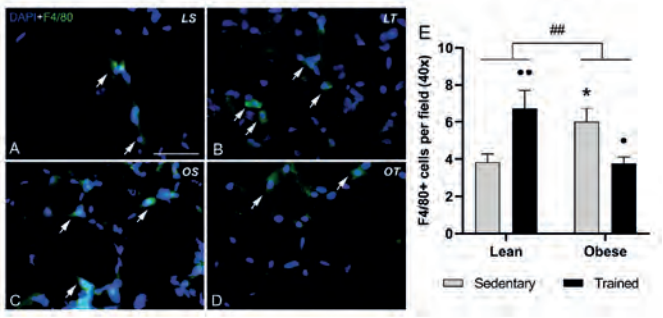


Figure 1: Representative images of F4/80+ (green) cells (macrophages, arrows) in white adipose tissue of lean sedentary mice (A), lean trained mice (B), obese sedentary mice (C) and obese trained mice (D). Nuclei were stained with DAPI (blue). Scale bar: 100 μm. Quantitative analysis showing the number of F4/80+ cells (E) in lean and obese mice (sedentary and trained groups). Each column shows the mean ± SEM of determinations in 10 random fields, performed in 9 animals from each sedentary group and 7 animals from each trained group. * p<0.05 vs. lean sedentary values; • p <0.05, •• p <0.01 vs. the corresponding sedentary values (Student's t-test); ## p<0.01 (two-way ANOVA). Scale bar: 100 μm. Abbreviations: LS, lean sedentary; LT, lean trained; OS, obese sedentary; OT, obese trained.

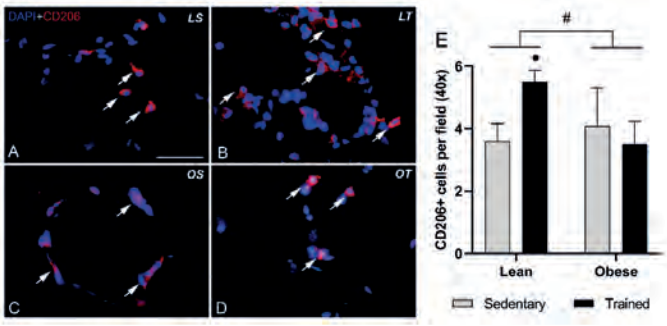


Figure 2: Representative images of CD206+ (red) cells (M2 macrophages, arrows) in white adipose tissue of lean sedentary mice (A), lean trained mice (B), obese sedentary mice (C) and obese trained mice (D). Nuclei were stained with DAPI (blue). Scale bar: 100 µm. Quantitative analysis showing the number of CD206+ cells (E) in lean and obese mice (sedentary and trained groups). Each column shows the mean ± SEM of determinations in 10 random fields, performed in 9 animals from each sedentary group and 7 animals from each trained group. • p <0.05 vs. the corresponding sedentary values (Student's t-test); # p<0.05 (two-way ANOVA). Scale bar: 100 µm. Abbreviations: LS, lean sedentary; LT, lean trained; OS, obese sedentary; OT, obese trained..

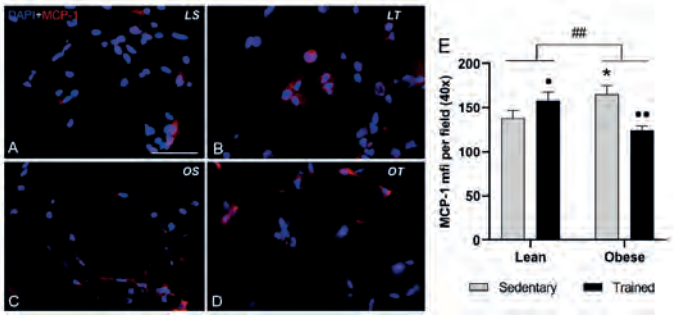


Figure 3: Representative images of MCP-1+ (red) expression in white adipose tissue of lean sedentary mice (A), lean trained mice (B), obese sedentary mice (C) and obese trained mice (D). Nuclei were stained with DAPI (blue). Scale bar: 100 µm. Quantitative analysis showing MCP-1 expression (E) in lean and obese mice (sedentary and trained groups). Each column shows the mean ± SEM of determinations in 10 random fields, performed in 9 animals from each sedentary group and 7 animals from each trained group. * p<0.05 vs. lean sedentary values; • p <0.05, •• p <0.01 vs. the corresponding sedentary values (Student's t-test); ## p<0.01 (two-way ANOVA). Scale bar: 100 µm. Abbreviations: LS, lean sedentary; LT, lean trained; OS, obese sedentary; OT, obese trained.

Crown-like structures in white adipose tissue

Notably, expression of MCP-1 in WAT was also localized to CLS (aggregated macrophages, surrounding individual adipocytes) in close relationship with F4/80 immunoreactive macrophages (as can be seen in novel immunochemistry images depicted in Figure 4A-D), both in lean and obese mice. Number of CLS was higher in sedentary obese animals vs. lean animals (p<0.001) (CLS formation was a rare event in lean sedentary mice) (Figure 4E). After the protocol of habitual exercise, a statistically significant different behaviour was observed between lean and obese groups (p<0.01): the presence of these structures was reduced in obese mice (p<0.05), while it was increased in lean mice (p<0.01) (Figure 4E), confirming that inflammatory responses can differ according to the individual's basal inflammatory set-point (42). Figures 4B, C and D show a representative CLS co-expressing the chemokine MCP-1 in close relationship with F4/80 immunoreactive macrophages. Another important feature in CLS was the presence of CD206+ cells (Figure 2) in lean and obese mice. These novel images reinforce the idea that CLS assessment constitutes an important approach for evaluating WAT inflammation involving both macrophage infiltration and MCP-1, as well as the pro- or anti-inflammatory effects of exercise in the context of their bioregulatory effects.

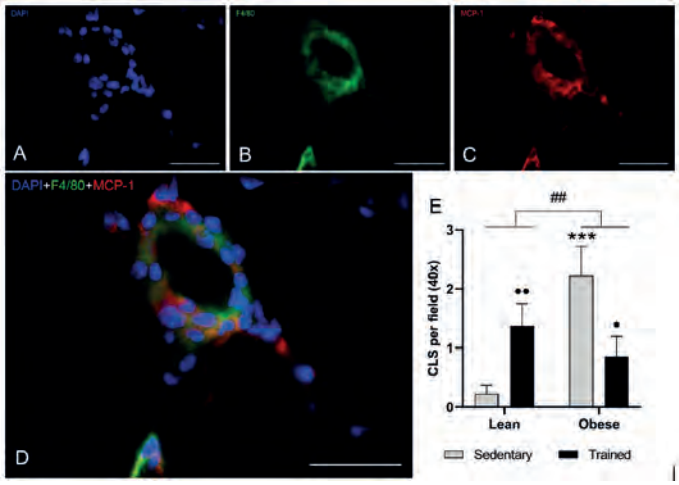


Figure 4: Novel, representative image of a crown-like structure (CLS) (D): F4/80+ (green) cells (macrophages) (B) surrounding an adipocyte in an obese sedentary mouse. This structure co-expressed the chemokine MCP-1 (red) (C). Nuclei were stained with DAPI (blue) (A). Scale bar: 50 µm. Quantitative analysis showing the number of crown-like structures (E) in lean and obese mice (sedentary and trained groups). Each column shows the mean ± SEM of determinations in 10 random fields, performed in 9 animals from each sedentary group and 7 animals from each trained group. *** p<0.01 vs. lean sedentary values; • p <0.05, •• p <0.01 vs. the corresponding sedentary values (Student's t-test); ## p<0.01 (two-way ANOVA). Scale bar: 50 µm

DISCUSSION

Physical exercise presents pleiotropic activity, affecting every physiological system, including the immune system. Although exercise strongly modulates all aspects of the immune response (41), it is particularly important in the modulation of the innate/inflammatory response (42), and the beneficial effects of moderate regular exercise on the inflammatory response are widely recognized to be mediated through anti-inflammatory mechanisms. Moderate regular exercise also has clear benefits in other aspects of the immune function, such as T cell function, antibody production, and phagocyte responses (e.g. phagocytic process) and therefore, it is associated with decreased susceptibility to infection (3, 11, 39). However, it is important to bear in mind that physical exercise does not cause a fixed and unvarying response, since it is a stress situation that causes an appropriate homeostatic response or adaptation to the changes that occur in each organism. This response depends on each individual's immunophysiological characteristics, with a special focus on the presence of inflammatory pathologies in which the basal inflammatory status is altered (11). In the same animal model of obesity used in the present study, results from our group have demonstrated that circulating monocytes from obese mice present a basal pro-inflammatory status (15) and that regular exercise elicits opposite responses in lean and obese mice: an

anti-inflammatory response in monocytes from obese mice and an overall pro-inflammatory effect in monocytes from lean mice (16). We have also found a differential immunophysiological behavior induced by exercise (pro-inflammatory in healthy individuals and anti-inflammatory in pro-inflammatory conditions) in the genetically obese Zucker rat (32) and in fibromyalgia—another inflammatory and stress-related pathology (4, 39).

Thus, inflammatory responses to exercise clearly differ according to the basal status or "inflammatory set-point". Exercise-induced activation of the innate and/or inflammatory responses in healthy people represents an immunophysiological adaptation in a situation of exercise-induced stress and vulnerability for the organism, being crucial for the defense against pathogenic attacks; while the anti-inflammatory effects induced by exercise in subjects with a pro-inflammatory set-point can be explained by the homeostatic adjustment preventing an excessive inflammatory response (42). This phenomenon has been coined "the bioregulatory effect of exercise", and it implies the regulation by exercise of the altered inflammatory and stress status depending on each individual's basal set-point, being anti-inflammatory mainly (or only) in the case of an elevated inflammatory status. This concept is defined as the reduction or prevention of an excessive inflammatory response together with the preservation or stimulation of the innate response (with optimal phagocytic and microbicidal activities) and the achievement of beneficial transitions between proinflammatory and classical monocytes and between M1 and M2 macrophages (42).

The present study examines the bioregulatory effects of exercise on the inflammatory response in the WAT of lean and obese animals, notably analyzing the effect of exercise on the formation of CLS, whose behaviour has shown them to be crucial for the assessment of the effects of exercise on the inflammatory response in the adipose tissue, confirming that inflammatory responses can differ according to the individual's basal inflammatory set-point. To this end, our approach involved the quantitative and qualitative evaluation of macrophage infiltration by using the antibodies F4/80, which identifies macrophages in the rodent tissue (47), and CD206, which has been recently proposed as the ideal marker to assess macrophage phenotype in WAT (37), since it has been shown by flow cytometry analysis that almost all CD206+ cells in WAT are macrophages, and not cells of other lineages, thus constituting exclusively M2 macrophage populations. Moreover, two clearly distinct cell subsets are found in the WAT macrophage population: most of the CD11c+ cells are CD206- (M1), whereas most of the CD11c- cells are CD206+ (M2) (14, 37, 48). Thus, by using this approach, we found that the effects of regular exercise on the inflammatory response observed locally in the WAT were congruent with the bioregulatory effects of exercise previously reported on systemic inflammatory responses. In this context, obese animals, which presented higher macrophage infiltration, showed significant reduction in these cells after the exercise protocol. Conversely, lean mice presented greater macrophage infiltration after the same exercise training protocol. Now, the next question was: what is the effect of exercise on CLS formation? Is it the same in obese and in lean individuals? The response clearly was that CLS results were in line with those regarding macrophage infiltration; that is obese sedentary mice presented a higher number of CLS than lean mice, and, remarkably, after the regular exercise protocol, CLS were also reduced in obese mice, whereas the presence of these structures increased in lean animals. In addition, another key finding was that the same pattern was observed in MCP-1 expression, with a reduction only in obese trained animals, which initially presented elevated expression levels of this chemokine at baseline. In fact, it is important to note that although MCP-1 is present throughout the WAT, it was fundamentally located in CLS. Therefore, these results altogether suggest that the effects of regular exercise on the local inflammatory response in the WAT seem to be anti-inflammatory only in obese subjects who present an elevated inflammatory status at baseline, while pro-inflammatory effects are observed in lean animals, in accordance with the bioregulatory effects of exercise. This novel bioregulatory effect in the adipose tissue involves CLS, representing a critical immunophysiological finding. CLS results are particularly relevant in supporting the bioregulatory effect of exercise, which is crucial in the potential exercise-induced modulation in the context of macrophage-mediated immunometabolism in the adipose tissue, such as macrophage recruitment to CLS (46, 55). To the best of our knowledge, this is the first time that bioregulatory effects of exercise have been reported in the adipose tissue, involving macrophages, MCP-1, and CLS. Most studies evaluating the influence of obesity or the effects of exercise on WAT inflammation do not evaluate structures or protein expression, nor do they use histological techniques and direct observation. Instead, flow cytometry in tissue lysates and mRNA detection methods are more commonly used. Furthermore, it is critical to take into account that CLS formation seems to be a better marker of WAT inflammation than indirect approaches or macrophage infiltration alone, since proliferation seems to occur predominantly in these structures (18). In fact, it has already been reported that 90% of macrophages in the adipose tissue of obese subjects are aggregated forming CLS (9, 36), thus constituting a major hallmark of inflammation in obesity. Additionally, the technique used in this work allowed us to observe the marked expression of MCP-1 in CLS, suggesting for the first time a potential role of this chemokine in the development of these structures, constituting a signal for macrophage recruitment or resident macrophage proliferation. Interestingly, MCP-1 is a promising drug target, although so far modulation of MCP-1 has not yet resulted in pharmacotherapies (12). Altogether, these findings provide a novel framework for understanding the physiological mechanisms that are involved in macrophage recruitment and function in WAT and for elucidating the underlying causes of obesity-associated WAT inflammation, including the influence of exercise on these aspects.

Apart from the bioregulatory effects of exercise, our findings regarding the influence of high-fat diet-induced obesity on macrophage accumulation and MCP-1 expression could be explained due to an infiltration of circulating monocytes into WAT and their subsequent differentiation into mature F4/80-expressing macrophages (53). Moreover, a major fraction of WAT macrophages undergoes cell division locally in obesity, leading to a local proliferation 'in situ' (1). Adipocytes and infiltrated macrophages can release signals such as MCP-1, causing increased monocyte influx (22, 53). MCP-1 has been extensively described as a potent chemokine attracting macrophages to the WAT in obese mice (22, 52), thus playing a very important role in promoting adipose tissue inflammation and insulin resistance (12). Moreover, in situ proliferation is partially driven by MCP-1 as well, also contributing to macrophage accumulation in the adipose tissue in obesity, in addition to blood monocyte recruitment. This suggests a positive correlation between MCP-1 expression and WAT macrophage accumulation in mice (1),

as corroborated by the present study, both by quantitative data analysis and by the novel image in the context of MCP-1 and CLS. Indeed, after exercise, MCP-1 expression and macrophage numbers decrease in obese mice and increase in lean mice. This is in accordance with previous studies showing that a high-fat diet increased MCP-1 gene or mRNA expression in the adipose tissue, with an exercise-induced reduction in MCP-1 (25, 27) and F4/80 gene or mRNA expression (51, 23, 27). However, other works could not find a reduction in MCP-1 mRNA expression in obese mice caused by exercise (23).

Thus, exercise is a crucial strategy in the management of low-grade inflammation in obesity. Controlling adipose tissue macrophage infiltration and proliferation has been proposed as a therapeutic target in the management of obesity, particularly to improve inflammation-related effects such as insulin resistance (35). However, controversial results on the modulation of glucose metabolism after the reduction of tissue macrophages have also been reported. Some studies have shown that a markedly reduced number of adipose tissue macrophages in MCP-1 knockout mice result in an amelioration of total insulin resistance (22), while mice overexpressing MCP-1 have increased numbers of infiltrating macrophages along with mildly increased insulin resistance (21, 22). Conversely, other studies have shown that, surprisingly, MCP-1 knockout mice presented increased glucose intolerance, even though these animals have lower adipose tissue macrophage proliferation (1, 20). This could be explained by the potential beneficial role of macrophages in increasing lipid storage or clearance of dead cells in adipose tissue (36). This does not happen in the present work (fasting glucose is not impaired after the exercise-induced macrophage numbers reduction in obesity), suggesting that an exercise-induced physiological reduction in macrophage infiltration is able to maintain glucose homeostasis, as opposed to genetic or pharmacological approaches aimed at reducing macrophage numbers to non-physiological levels that might negatively affect the physiological immune functions of macrophages in tissues (35). However, at least in our protocol of exercise, the exercise-induced reduction in macrophage accumulation does not seem to be enough to achieve a decrease in fasting glucose levels, although it does not increase them either, as mentioned before. It is important to highlight that, surprisingly, the pro-inflammatory effects in trained lean mice does not seem to be accompanied by an impaired metabolic response (i.e. glycemic and lipid profile), reinforcing the potential bioregulatory physiological role in stimulating the immune response, without detrimental metabolic effects. In fact, several parameters such as HDL-C, cLDL-C and triglycerides significantly improve in lean mice after exercise, and the same parameters also improve in trained obese mice. Thus, exercise-induced inflammatory changes in the WAT are accompanied by metabolic improvement, or at least, lack of impairment.

After establishing all the aforementioned findings regarding macrophage infiltration, CLS formation, and MCP-1 expression in our animal model and in response to the protocol of regular exercise, our next question was whether macrophage inflammatory phenotype (evaluated through CD206 expression) could be altered both in obesity and in response to exercise. No baseline differences in isolated CD206+ macrophages were found between lean and obese mice in the present study. It is well-known that the WAT of obese animals presents a preferential recruitment of M1-type macrophages and/or a phenotypic switch of adipose tissue macrophages towards the M1 phenotype, increasing the proportion of M1 to M2 macrophages; while WAT of lean individuals contains fewer macrophages, which present mainly a M2 phenotype (6, 29). Several studies show differing results and should be examined closely for the way cell numbers and functions are expressed, especially when immune cell numbers are determined by flow cytometric analyses or gene expression. Some studies suggest that obesity only appears to increase the numbers of the M1 population while M2 numbers remain unchanged, thus the obesity-induced changes in macrophage phenotypes are mainly due to increases in the M1 subpopulation (49, 26), which is in accordance with our results showing similar numbers of M2 macrophages in lean and obese mice. Other authors have suggested that, considering the dramatic increase of infiltrated macrophages in obesity, the absolute number of both M1- and M2-polarized macrophages increases (6), while others show an increase in M1 and a decrease in M2 macrophages (23). In any case, very few studies have specifically characterized WAT immune cell phenotypes following exercise training (56). Gleeson et al. (2011) proposed that the anti-inflammatory effects of exercise rely on several mechanisms, including the inhibition of monocyte and macrophage infiltration and the phenotypic switching of macrophages within WAT (17). In the seminal work by Kawanishi et al. (2010), exercise training reduced the elevated CD11c (M1) mRNA expression, but increased the low CD163 (M2) mRNA expression in obese mice, suggesting that exercise was associated, not only with a reduction in total WAT macrophage content, but also with an M1–M2 phenotype switch in obesity (23). A recent study found similar results, proposing that exercise increased M2 macrophage polarization (2). Conversely, other observations in obese animals showed that regular exercise suppressed the elevated CD11c gene expression, and also attenuated the elevated CD206 and arginase-1 mRNA expression (27). However, no significant changes were found in lean exercised animals in any of these studies. In the present paper, we focused on M2 macrophages since they can positively alter the outcome of obesity due to their beneficial function in apoptotic cell clearance, tissue repair, and remodeling in the WAT (6). We observed that the population of CD206+ macrophages increased only in lean mice after exercise. This might occur in response to the post-exercise increase in macrophages in lean animals, as a way to counteract the potential pro-inflammatory effects due to higher macrophage accumulation. Thus, although the presence of infiltrating or proliferating macrophages in the adipose tissue of trained lean mice increases, macrophages with an anti-inflammatory profile increase as well. On the other hand, in trained obese animals the number of macrophages decreases while the number of CD206+ macrophages is maintained. This might reflect a reduction of macrophage accumulation mainly at the expense of pro-inflammatory M1 macrophages, in accordance with the anti-inflammatory effects of exercise observed in this group of obese animals.

It is important to note that circulating monocytes from this animal model of obesity have shown a pro-inflammatory profile, including cytokine expression and phenotype markers (15), while peritoneal macrophages did not seem to present a higher inflammatory profile (30). It can be hypothesized that greater adipose tissue infiltration of macrophages with a pro-inflammatory profile leaves less activated cells in the peritoneal cavity (as non-infiltrated macrophages). In fact, it has already been reported by Forner et al. (1994) that peritoneal macrophages from old animals (in which chronic, low-grade inflammation develops due

to advanced age, "inflammaging"), present increased adherence capacity and reduced chemotaxis capacity (13). Nevertheless, when evaluating macrophage infiltration and their inflammatory profiles, it is crucial to consider that WAT inflammation depends not only on immune cell infiltration per se, but also on the complex immune cell-adipocyte interactions (43), thus the importance of direct observation of major inflammatory structures such as CLS and their association with inflammatory cells and mediators that might have a key role in the development of adipose tissue disfunction in obesity. Therefore, the evaluation of CLS on the context of anti-inflammatory strategies such as exercise seems fundamental.

Since the phenotype study was limited to M2 macrophage analysis, it was not possible to assess M1 macrophages. Future studies should focus on the analysis of the bioregulatory effects of exercise on this macrophage population, together with other chemokines, inflammatory mediators, and innate immune function in the WAT. Moreover, further studies are needed to elucidate the influence of obesity and exercise on the mechanisms by which different macrophage subtypes and inflammatory mediators regulate the cell interplay, tissue remodeling, and metabolic functions within the adipose tissue. Notwithstanding these limitations, our study clearly contributes to the understanding of the effects of exercise on the inflammatory response in the WAT, both in healthy and pro-inflammatory conditions from a novel approach.

CONCLUSIONS

High-fat diet-induced obesity is associated with greater MCP-1 expression, macrophage accumulation, and crown-like structure presence. Regular exercise reduced macrophage accumulation, MCP-1 expression, and crown-like structure presence in obese mice; while it increased macrophage and crown-like structure presence, MCP-1 expression, and M2 polarization in lean mice. A novel finding was that MCP-1 was associated with the proliferation of crown-like structures. Altogether, these results confirm, for the first time, the "bioregulatory effect of exercise" in the adipose tissue, with the particular immunophysiological relevance of crown-like structure formation: reducing inflammation in individuals with an elevated inflammatory set-point, but stimulating this response of the immune system in healthy individuals. The present results contribute to our understanding of adipose tissue inflammation biology and may lead to novel therapeutic strategies and specific targets for potential intervention to prevent or treat obesity-induced adipose tissue inflammation.

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