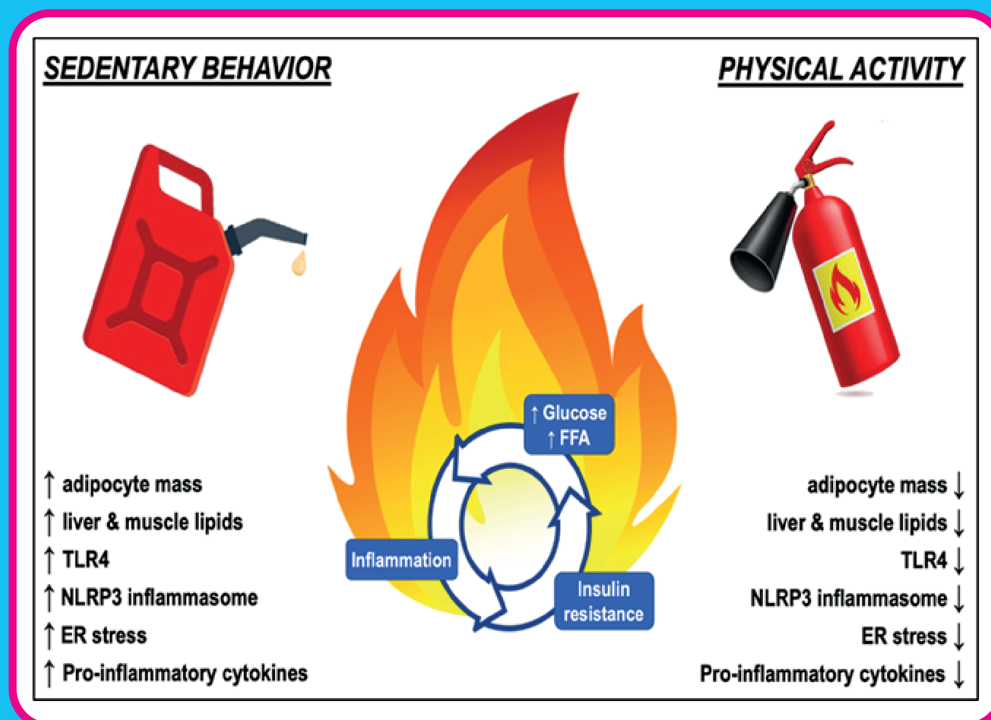
EXERCISE IMMUNOLOGY REVIEW



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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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Acute effects of heavy resistance exercise on biomarkers of neuroendocrine-immune regulation in healthy adults: a systematic review

Simon Haunhorst[1,3], Wilhelm Bloch[2], Miriam Ringleb[1], Lena Fennen[1], Heiko Wagner[1], Holger H.W. Gabriel[3], Christian Puta[3,4]

[1] Department of Movement Science, University of Münster, Münster, Germany
[2] Department for Molecular and Cellular Sports Medicine, Institute for Cardiovascular Research and Sports Medicine, German Sport University Cologne, Cologne, Germany
[3] Department of Sports Medicine and Health Promotion, Friedrich-Schiller-University Jena, Jena, Germany
[4] Center for Interdisciplinary Prevention of Diseases related to Professional Activities, Friedrich-Schiller-University Jena, Jena, Germany

ABSTRACT

Background: The nervous system integrates the immune system in the systemic effort to maintain or restore the organism's homeostasis. Acute bouts of exercise may alter the activity of specific pathways associated with neuroendocrine regulation of the immune system.
Objective: To examine the acute effects of heavy resistance exercise on biomarkers of neuroendocrine-immune regulation in healthy adults.
Methods: A systematic literature search was conducted using PubMed, Cochrane Controlled Trials Register, Web of Science and SportDiscus with no date restrictions up to March 2021. Clinical trials in English or German were included if they measured the blood plasma or serum concentrations of specific biomarkers of neuroendocrine-immune regulation (adrenaline, noradrenaline, acetylcholine, vasoactive intestinal peptide (VIP), cortisol, growth hormone, calcitonin gene-related peptide (CGRP), substance p, serotonin, brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) or glia-derived neurotrophic factor (GDNF)) in a resting state prior to and no later than 60 minutes after an acute bout of heavy resistance exercise in healthy adults.
Results: 7801 records were identified through literature search, of which 36 studies, with a total of 58 intervention groups, met the inclusion criteria. Evidence was found that an acute bout of heavy resistance exercise increased the levels of adrenaline (median: 185%), noradrenaline (median: 113%) and GH (median: 265%) immediately after the exercise. Mixed results were found for cortisol (median: 0%), suggesting that its response might be more sensitive to the configuration of the exercise scheme. The limited evidence regarding the effects on BDNF and ACTH allows no firm conclusions to be drawn about their response to heavy resistance exercise. The vast majority of the included studies reported a return of the biomarker concentrations to their baseline value within one hour after the termination of the exercise bout. No studies were identified that investigated the response of acetylcholine, VIP, CGRP, substance p, serotonin, NGF or GDNF to heavy resistance exercise.
Conclusions: A bout of heavy resistance exercise alters the circulating concentrations of selected biomarkers of neuroendocrine-immune regulation. Both subject characteristics, such as sex as well as exercise parameters, such as rest intervals appear to have the potential to influence these effects.

Keywords: Resistance exercise; nervous system; immune system; neuroendocrine reaction; immune regulation

Corresponding author:
Simon Haunhorst
Department of Movement Science
Westfälische Wilhelms-Universität Münster
Horstmarer Landweg 62b
48149 Münster, Germany
E-Mail: shaunhor@uni-muenster.de

PD Dr. Christian Puta
Department of Sports Medicine and Health Promotion
Friedrich-Schiller-Universität Jena
Wöllnitzer Straße 42
07749 Jena, Germany
E-Mail: christian.puta@uni-jena.de

INTRODUCTION

The nervous system and the immune system are vital for the organism's survival and are in constant communication in pursuit of maintaining or restoring homeostasis [21, 112]. The brain integrates the immune system in the systemic effort to effectively cope with stressors such as invasive agents or tissue injuries [100].

The brain's regulative control provides distinct advantages for the immune system. Specifically, the nervous system's ability to transmit information at rapid speeds and to sense pathogens or tissue damage-associated factors ensures a fast and effective immune response [100]. Additionally, the brain is constantly monitoring the internal and external environment. It is able to synchronize the immune system with other systems and processes it is dependent on, like blood flow and the digestive system [100]. By combining information about the external environment gathered by the sensory organs, the brain can anticipate potential threats to the body's homeostasis and prepare the immune system accordingly [1, 15].

The homeostatic internal milieu is however not only challenged by viruses or bacteria, but also by physical exercise. Depending on the duration and intensity, exercise constitutes a stimulus that demands physiological and psychological resources [87]. The immunological response to the stressor is predominantly characterized by a short-term redistribution of immune cells into the circulation, their infiltration of tissues and a rise in the circulating levels of cytokines [30, 31, 35, 74, 87].

Previous studies indicate that this integration of the immune system into an orchestrated, systemic stress response is achieved either through the control of blood flow, metabolic activity or muscle action [24, 90, 91, 100] or directly via the efferent arms of several neuro-immune pathways. The sympathetic, parasympathetic, somatosensory, neuroendocrine and neurotrophic pathways act as interfaces between the nervous system and the immune system [29, 36, 50, 57, 58, 104, 110, 119]. Measuring specific biomarkers in the peripheral blood that are associated with the activity of these pathways such as adrenaline, noradrenaline, acetylcholine, cortisol, serotonin or brain-derived neurotrophic factor (BDNF), to name a few, allows a conclusion to be drawn about their involvement in the body's stress response and nature of the neuroendocrine-immune regulation. Although being primarily used for communication within the neuroendocrine system, the expression of specific receptors for these biomarkers on leukocytes lays the foundation for the functional connectivity between the nervous system, endocrine system and the immune system. Specific effects upon receptor binding include the exercise-induced redistribution of T-lymphocytes within lymphoid and non-lymphoid organs, mediated by α- and β-adrenoceptor signaling [71]. The general mobilization pattern of lymphocytes during exercise is related to the differential expression of β-adrenergic receptors on lymphocytes (Natural killer cells > CD8+ T-cells > B-cells > CD4+ T-cells) [11, 70, 115]. Furthermore, the noradrenaline-mediated CD4+ T-cell differentiation [21] or the acetylcholine-mediated attenuation of inflammation through the inhibited secretion of TNF, IL-1β, IL-6 and IL-18 by macrophages [104] are among the reported effects.

In recent years, central neuronal factors, such as BDNF, nerve growth factor (NGF) or serotonin that were previously associated with neurological processes gained increasing attention in the context of immunoregulation, as well. BDNF for example has been described to be an anti-apoptotic survival factor for B- and T-cells and to promote glial cell proliferation [53, 102, 125].

The response of the biomarkers of neuroendocrine-immune regulation to acute exercise stress is multifaceted and dependent on several exercise program variables like volume, intensity, duration and mode [18, 67]. For resistance exercise for instance, it has been demonstrated that, in general, protocols with a high intensity, high volume and short rest intervals cause the greatest elevations of circulating biomarkers [30]. Especially, increments of classical stress hormones such as adrenaline, noradrenaline and cortisol as well as of anabolic hormones such as growth hormone and neurotrophins have been documented in response to acute bouts of resistance exercise [28, 30, 67]. Literature comparing this reaction directly to endurance exercise is sparse. Evidence exists suggesting that the direction of the effects is similar, the magnitude might however differ owing to the fact that resistance and endurance exercise differ in terms of muscle fiber recruitment and hemodynamics [44], Additionally, the metabolic pathways used for energy production during exercise could give an indication for the biomarker response. Existing evidence indicates that at similar exercise intensities, higher rates of anaerobic glycolysis (e.g., during resistance exercise) lead to greater cortisol increments due to its relationship with lactate concentrations [3, 113].

As resistance exercise with heavy loads has been described to be more demanding with regards to the neuromuscular activity than with light loads [122], approaching changes in neuroendocrine-immune pathway activity from a standpoint of heavy resistance exercise appears to be worthwhile. However, to the author's knowledge, no systematic review to date has characterized the alterations in the activity of these pathways of neuroendocrine-immune regulation in response to an acute bout of heavy resistance exercise in healthy adults.

Therefore, the aim of this systematic review is to examine the acute effects of heavy resistance exercise on selected biomarkers of neuroendocrine-immune regulation in healthy adults.

METHODS

The protocol for this systematic review was prospectively registered with the Open Science Framework (https://osf.io/ebr4k). Amendments to the protocol can be accessed via the corresponding project (https://osf.io/a8b23/). It was conducted and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Statement [72].

Eligibility criteria

The eligibility criteria were formed a priori using the PICOS (participants, intervention, comparators, outcomes, and study design) approach.

Inclusion criteria were defined as follows: (1) participants: a cohort of healthy adults (18 years of age or older) (2) intervention: single session of heavy resistance exercise, defined as concentric and eccentric muscle actions to overcome externally applied resistance with a load of more than 80% of the individual's one repetition maximum (1RM) or

100% of the 1-8RM (3) outcomes: blood plasma or serum measurements of at least one of the following biomarkers of neuroendocrine-immune regulation at rest and follow-up (within 60 minutes after termination of exercise): Adrenaline, noradrenaline, acetylcholine, vasoactive intestinal peptide (VIP), cortisol, growth hormone, CGRP, substance p, serotonin, BDNF, nerve growth factor (NGF) and glia-derived neurotrophic factor (GDNF) (4) study design: clinical trials (5) studies published in a peer-reviewed journal in English or German.

Exclusion criteria were defined as follows: (1) a cohort with subjects below 18 years of age or with health problems (e.g., diabetes mellitus or multiple sclerosis) (2) resistance exercise that was combined with other treatment modalities that could alter the physiological response to exercise (e.g., blood flow restriction or pharmacological supplementation), that used a load that was lower than 80% of the 1RM and/or that was used as a follow-up measurement in a training intervention program (3) no baseline measurement or a follow-up measurement that was conducted later than 60 minutes after termination of the exercise (4) reviews, cross-sectional or retrospective longitudinal study designs, meeting abstracts and conference proceedings, letters to the editor or records with no identifiable abstract (5) studies published in other languages than English or German.

Literature search

The literature search was conducted in March 2021 in the electronic databases PubMed, Web of Science, Cochrane CENTRAL Library and SportDiscus with no restrictions on date, publication type or language. The search terms were collected through experts' opinion, literature scoping and related vocabulary. They covered the following domains: resistance training, biomarkers and blood sample (serum and plasma). The exact search syntax for each database can be accessed via the aforementioned link to the Open Science Framework. In order to identify further studies the reference lists of included studies were examined and key journals hand searched.

Study selection

The identified records were downloaded from the electronic databases and managed in Zotero (version 5.0.96.2). After the detection and deletion of duplicates, the records were exported to Rayyan (https://rayyan.qcri.org), a free web-based platform that enables a collaborative record management. In Rayyan relevant studies were independently selected in a two-stage process by SH and MR. In the first stage, titles and abstract were screened. The studies that did not meet the eligibility criteria were excluded. In the second stage, the full-text articles of the remaining studies were accessed. Studies that were considered eligible after screening of the full text were included into the review process. Cases of disagreements were solved by discussion at the end of both stages. If necessary, a third reviewer was consulted for clarification.

Reasons of exclusion in the second stage were documented and can be observed in Figure 1 together with all other information on the selection process.

Data extraction

The data extraction was performed by SH and verified by a second reviewer. Cases of disagreements were solved by discussion. The following data items were extracted from the included studies using a standardized form in Microsoft Excel: the authors, year of publication, pre-post intervention group sample size and participant characteristics including sex, age, height, weight and resistance training experience. Participants were deemed inexperienced if their absence of experience was explicitly stated or if they were not involved in any form of resistance exercise within the last three months prior to testing. Furthermore, the exercises performed, training volume and intensity, time of day, biomarkers measured, follow-up measurement intervals, blood samples used, analysis methods, as well as the main outcome related findings and baseline and follow-up concentrations of the biomarkers were extracted. If biomarker concentrations were not provided in the studies, the first and last authors were contacted via their institutional mail addresses. The WebPlotDigitizer digitization program (https://automeris.io/WebPlotDigitizer/) was used to extract plotted data if authors did not respond within one month.

Study quality

The risk of bias of the included studies was independently assessed by SH and CP using a modified version of the quality appraisal tool developed by Brook Galna and colleagues [34]. The tool consists of 14 questions focusing on the external validity, internal validity and reproducibility of the study. Each question was scored on a scale of zero to one, where one indicates high quality and zero low quality. For the purpose of this review, the fifth item of the original tool was left out, taking the different methodological approaches of the studies included in the present review and the review by Galna et al. into account.

Data synthesis

The results of the literature search, the study and sample characteristics and risk of bias assessment were summarized in figures and tables. Given the fact that the included studies did not provide standard deviations, variances, precise p-values or effect estimates, the effect of heavy resistance exercise on the biomarker levels was computed as the percentage change from a resting baseline value to the immediate post-exercise value. In accordance with the Cochrane Handbook for Systematic Reviews of Interventions [81], the narrative summary of the effects was complemented by the effect distributions (median, range, interquartile range) for each biomarker computed in R Studio (Version 1.4.1106). Furthermore, the magnitude and direction of the effects on the study levels were displayed using bar charts.

RESULTS

Study selection

A total of 7801 records were identified through database searching (PubMed: 1404, Web of Science: 4458, Cochrane: 971, SportDiscus: 968). After the removal of duplicates, 5726 studies were screened by titles and abstracts. Of these, 5678 records were removed, leaving 48 eligible for full-text screening. A further 14 articles were removed, and 2 records

were added through hand-searching key journals and reference lists, resulting in 36 articles with a total of 58 pre-post intervention groups that met the inclusion criteria and were included into the qualitative synthesis. The studies by Church et al. [13] and Mangine et al. [75] as well as by Rahimi et al. [96] and Rahimi et al. [97] were published based on the same experiments, respectively. Since they are separate publications reporting the effects on different outcome measures, they were not treated as duplicate studies. The search and study selection process are detailed in Figure 1.

All the resistance exercise interventions conducted in the 36 studies included a lower body exercise, of which 30 (83%) included multi-joint lower body exercises. 20 studies (56%) added upper body exercises to the protocol. The exercise sessions were predominately (56%) conducted in the morning hours. The session volume, expressed as the total number of repetitions, ranged between 8 and 280. All studies, except for five that used the high-performance liquid chromatography (HPLC), used immunoassays to analyze blood samples, with the enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) as the predominant choices. The reported intra-assay and inter-assay coefficients of variation were below 10%.

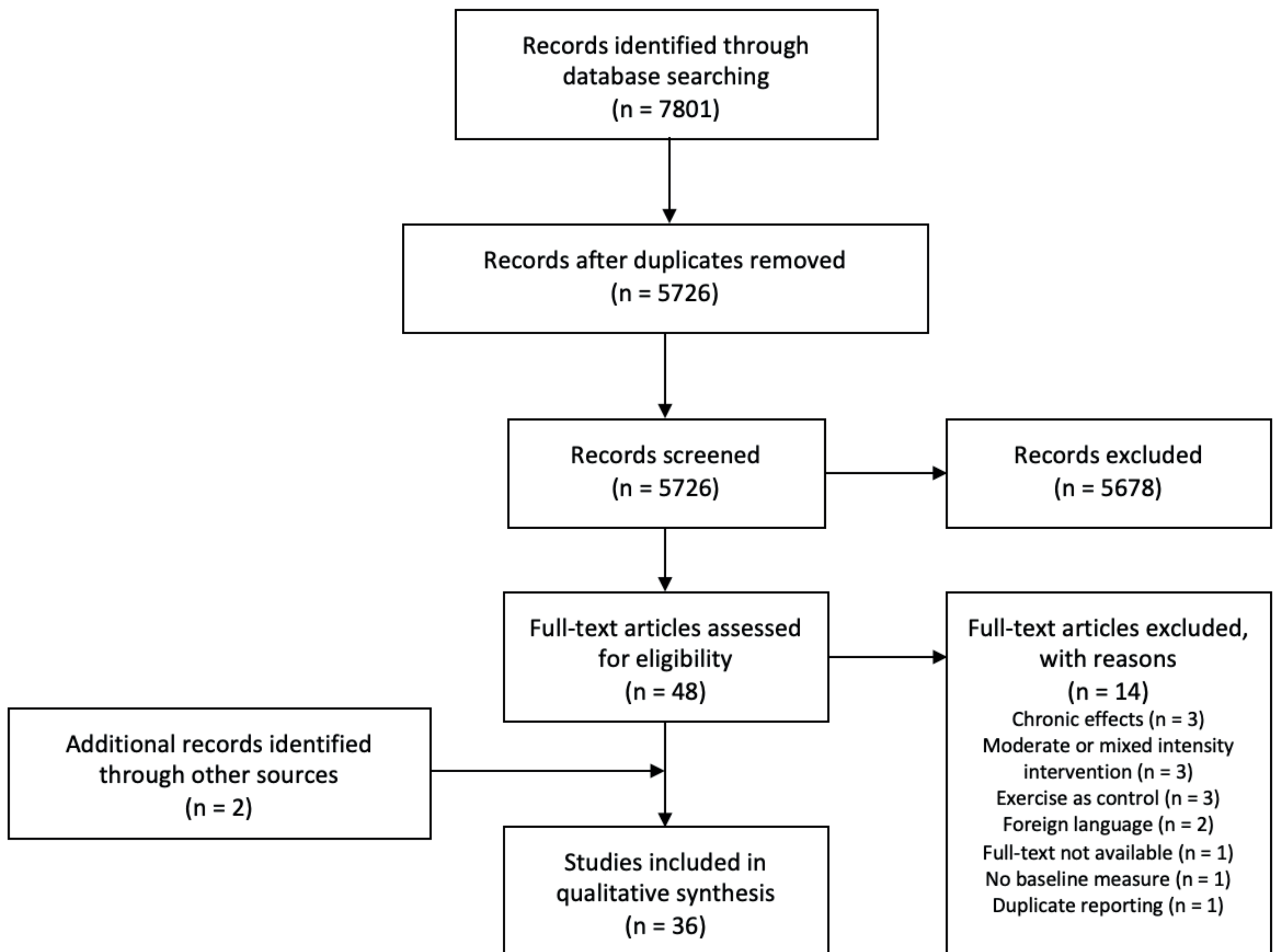

Figure 1. Flow diagram of literature search and study selection

Study characteristics

A detailed summary of the characteristics of the included studies is presented in Table 1. All articles were published in English between the years 1990 and 2020. Seven studies (19%) included a sample of men and women, while 25 studies (69%) recruited only men and three studies (8%) only women. One study (3%) did not report the sex proportion. The mean age of the subjects in the included intervention groups was 27.5 years, with a range from 18 to over 72 years. The participants of eight studies (22%) were deemed inexperienced with regards to resistance training, while 21 studies (58%) included participants with some form of resistance training experience, either as recreational (n=19) or professional athletes (n=3). One study (3%) included both inexperienced subjects and professional athletes and five studies (14%) did not report the resistance training experience of their participants.

Quality assessment

The studies included into this systematic review stated their research aims clearly, with studies lacking detail or clarity in only two cases [52, 82]. The participants included in the studies were detailed sufficiently, the sampling and recruitment methods were however described unsatisfactorily, compromising the ability to repeat the studies appropriately. The repeatability of the study procedure was impaired in several cases due to the fact that it was not detailed at which time of the day the participants were tested and if they were sober or postprandial. Overall, the described methodology was able to answer the research question adequately. The

Reference	Design	N (m/f)	Age (years) M±SD	Resistance training level	Exercises	Volume Sets x Reps; Rest (mins)	Intensity	Time of day	Hormone	Sample	Analysis method	Follow-up measurement (mins)	Main outcome related findings
Bosco et al. (6)	Pre-post	12 (6/6)	m: 23.3±2.4 f: 23.0±2.7	NR	Half squat, full squat	6 x 16; 8	80% 1RM	10:00-12:30	C	Serum	RIA	0	1% decrease of C 0 mins post in women; 35%* decrease of C in men
Burley et al. (9)	Pre-post with control	24 (24/0)	21.4±1.9	Inexperienced	Leg press, chest press, lat. pull-down, shoulder press	3 x 10; 2	80% 1RM	08:00 or 18:00	C	Serum	ELISA	5	No change of C 5 mins post in morning, 6% increase in evening group; sig. higher C levels in morning session compared to evening
Church et al. (13)	Pre-post	10 (10/0)	22.6±2.3	Experienced	Back squats, deadlifts, leg press, lat. pull-down, barbell rows, barbell curls	4 x 3-5; 3	90% 1RM	NR	BDNF	Plasma	ELISA	0, 30, 60	62%* increase of BDNF 0 mins post; stayed sig. elevated at 30 and 60
Cui et al. (19)	Pre-post	15 (NR)	18.9±0.1	Inexperienced	Bench press, squat, lat. pull-down, overhead press, standing dumbbell curl	4 x 6; 3	90% 1RM	16:00	C	Plasma	CLIA	0, 60	38%* increase of C 0 mins post; no sig. change between 0 and 60 mins post
Dalbo et al. (20)	Pre-post	22 (22/0)	21±1 68±1	NR	Squats, leg press, leg extensions	3 x 10; 3	80% 1RM	06:00-09:00	C	Serum	ELISA	5	114%* increase of C in young and 167%* in old men 5 mins post
Doma et al. (23)	Pre-post	14 (14/0)	24.0±3.9	Inexperienced	Squats, single-leg leg press, leg extension, leg curls	3 x 6; NR	95% 6RM	NR	C	Serum	FIA	0	Sig. increase of C 0 mins post during first session; no sig. change during second visit 2 weeks later
Flynn et al. (27)	Pre-post with control	15 (0/15)	72.6±3.5	Inexperienced	Leg extension, leg curl, plantar flexion, dorsiflexion, leg abduction, leg adduction, hip extension, hip flexion	2-3 x 8; 2	80% 1RM	08:00	C	Serum	RIA	0	33%* decrease of C 0 mins post
Fragala et al. (31)	Pre-post with control	15 (8/7)	m: 24.6±5.1 f: 22.1±3.1	Experienced	Back squats	6 x 5; 3	90% 1RM	05:30-08:00	A, NA	Plasma	HPLC	0, 60	233%* and 424%* increase of A and NA in men, 131%* and 409%* increase of A and NA in women 0 mins post with no sig. gender difference; returned to baseline 60 mins post
French et al. (33)	Pre-post with control	10 (10/0)	23±2	Experienced	Smith's squats	6 x 10; 2	80% 1RM	07:00-10:00	A, NA	Plasma	RIA	0, 5	185%* and 149%* increase of A and NA 0 mins post; sig. anticipatory increase of A and NA immediately before compared to 60 mins before exercise
Goto et al. (40)	Pre-post	8 (8/0)	20-23	Experienced	Bilateral knee extension	5 x 5; 3	90% 1RM	NR	GH	Serum	RIA	5, 15, 30, 60	Small increase of GH post exercise which is not reported to be significant
Goto et al. (41)	Pre-post	6 (6/0)	24.3±0.4	Experienced	Bilateral knee extension	5 x NR; 1	80% 1RM	08:00-12:00	A, NA C, GH	Plasma Serum	HPLC RIA	5, 15, 30	73%* and 113%* increase of A and NA 5 mins post; returned to baseline 15 mins post 18% and 882% increase of C and GH 5 mins post; decrease of C, further increase of GH during recovery
Goto et al. (39)	Pre-post	9 (9/0)	24±0.2	Experienced	Bilateral knee extension	4 x 9; 1	80% 1RM	08:00-12:00	A, NA C, GH	Plasma Serum	HPLC RIA	0, 15, 30	262%* and 314%* increase of A and NA 5 mins post; returned to baseline 15 mins post 16% and 12% increase of C and GH 5 mins post; decrease of C, further increase of GH during recovery
Häkkinen and Pakarinen (45)	Pre-post with control	10 (10/0)	29.7±8	Professional athletes	Back squat	20 x 1; 3	100% 1RM	17:00-19:00	C, GH	Serum	RIA	0, 60	7% decrease of C 0 mins post; sig. decrease from 0 to 60 mins post 361%* increase of GH 0 mins post; sig. decrease from 0 to 60 mins post
Jablu and Hosseini (49)	Pre-post with control	10 (0/10)	54.3±3.7	NR	Supine chest press, lat. pull-down, leg press, biceps curl, triceps pushdown, leg curl, leg extension. shoulder press	3 x 10; 1	80% 1RM	08:00-10:30	C	Serum	CLIA	0, 15	35% decrease of C 0 mins post
Jin et al. (52)	Pre-post	10 (10/0)	20.8±1.4	NR	Exercise circuits: bench press, seated cable row, sit-up, leg extension, leg curl	5-10 x 8; 0.5	85% 1RM	NR	C	Serum	RIA	0, 30	26%* increase of C 0 mins post; stayed sig. increased 30 mins post
Kokalas et al. (60)	Pre-post with control	6 (6/0)	20±1.6	Professional athletes	Bench pulls in prone position, leg press in sitting position, rowing from standing position	6 x 6; 2	85-100% 1RM	08:00-09:00	C, GH	Serum	ELISA	0	36% decrease of C and 2633% increase of GH 0 mins post, significance level NR

Reference	Design	N (m/f)	Age (years) M±SD	Resistance training status	Exercises	Volume Sets x Reps; Rest (mins)	Intensity	Time of day	Hormone	Sample	Analysis method	Follow-up measurement (mins)	Main outcome related findings
Kraemer et al. (65)	Pre-post	9 (9/0)	24.7±4.3	Experienced	Bench press, bilateral leg extensions, military press, sit-ups, seated rows, lat. pull-downs, arm curls, leg press	3-5 x 5; 3 or 1	100% 5RM	NR	GH	Serum	RIA	0, 5, 15, 30, 60	252%* and 330%* increase of GH in 3 and 1 mins rest protocol 0 mins post; returned to baseline 5 and 15 mins post in 3 and 1 mins rest protocol respectively Increase sig. greater with shorter rest
Kraemer et al. (66)	Pre-post	16 (8/8)	m: 24.7±4.5 f: 23.1±3.3	Experienced	Bench press, bilateral leg extensions, military press, sit-ups, seated rows, lat. pull-downs, arm curls, leg press	3-5 x 5; 3	80-95% 1RM	08:00-10:00	GH	Serum	RIA	0, 5, 15, 30, 60	75% increase of GH 0 mins post in men; further increase until 30 mins post; returned to baseline 60 mins post 26% decrease of GH 0 mins post in women; further decrease during recovery
Kraemer et al. (62)	Pre-post	9 (0/9)	24.1±4.3	Experienced	Bench press, bilateral leg extensions, military press, sit-ups, seated rows, lat. pull-downs, arm curls, leg press	3-5 x 5; 3 or 1	100% 5RM	08:00-10:00	C, GH	Serum	RIA	0, 5, 15, 30, 60	35% increase and no change of C in 3 and 1 mins rest protocol 0 mins post; no sig. change in both protocols during recovery 27% decrease and 24% increase of GH in 3 and 1 mins rest protocol 0 mins post; decrease below baseline in both protocols during recovery
Kraemer et al. (61)	Pre-post	8 (8/0)	24.7±1.6	Experienced	Bench press, bilateral leg extensions, military press, sit-ups, seated rows, lat. pull-downs, arm curls, leg press	3-5 x 5; 3 or 1	100% 5RM	NR	C	Serum	RIA	0, 5, 15	1% and 9% increase of C in 3 and 1 mins rest protocol 0 mins post; decrease below baseline in both protocols during recovery
Kraemer et al. (69)	Pre-post	21 (13/8)	m: 25.3±3.2 f: 20.6±1.5	Inexperienced	Squat, leg press, knee extension	3 x 6-8; 2	100% 6-8RM	NR	C, GH	Serum	RIA	0, 5	50%* and 1159%* increase of C and GH 0 mins post in men 7% and 265%* increase of C and GH 0 mins post in women
Kraemer et al. (63)	Pre-post	19 (19/0)	Athletes: 24.7±3.8 Untrained: 26.6±5.9	Professional athletes or Inexperienced	Bilateral leg press	1 x 20-21	80% 1RM	NR	A, C, NA	Plasma	HPLC, RIA	0,5	70%* and 95%* increase of A and NA and 21% decrease of C 0 mins post in athletes 68%* and 58%* increase of A and NA and 5% decrease of C 0 mins post in untrained subjects
Mangine et al. (75)	Pre-post	15 (15/0)	24.7±3.4	Experienced	Back squats, deadlifts, leg press, lat. pull-down, barbell rows, barbell curls	4 x 3-5; 3	90% 1RM	NR	C, GH	Serum	ELISA	0, 30, 60	22%* decrease of C and 119% increase of GH 0 mins post; C stayed below and GH decreased below baseline during recovery
Marston et al. (77)	Pre-post	16 (11/5)	m: 25±1.3 f: 23.2±1.3	Inexperienced	Bench press, lat. pull-down, leg press, leg extension, seated row, military press, dumbbell arm curl	5 x 5; 2	100% 5RM	NR	BDNF	Serum	ELISA	0, 30	1% decrease of BDNF 0 mins post, decrease below baseline during recovery
Marston et al. (76)	Pre-post	14 (NR)	55.2±6.8	Inexperienced	Bench press, leg press, lat. pull-down, leg curl	5 x 5; 2	85% 1RM	NR	BDNF	Serum	ELISA	0, 30	3% increase of BDNF 0 mins post, decrease below baseline during recovery
McMurray et al. (82)	Pre-post with control	8 (8/0)	18-30	Experienced	Circuit: Leg press, bench press, leg extension, lat. pull-down, leg curl, military press	3 x 6-8; NR	80% 1RM	18:00	C, GH	Plasma	RIA	0, 20, 40, 60	17% increase of C 0 mins post; peak at 20 and decrease below baseline at 60 mins 2835%* increase of GH 0 mins post; stayed sig. elevated and returned to baseline 60 mins post
Pareja-Blanco et al. (89)	Pre-post	10 (10/0)	22.1±3.5	Experienced	Smith's bench press, Smith's squat	3 x 8, 6 or 4; 5	80, 85 or 90% 1RM	10:00	C, GH	Plasma	ECLIA	5	Sig. decrease of C 5 mins post in 4 reps without failure protocol; no sig. change in all other protocols No sig. change of GH 5 mins post regardless of protocol
Pullinen et al. (94)	Pre-post	17 (9/8)	m: 29±3 f: 27±4	Experienced	Bilateral knee extension	1 x 8-10	80% 1RM	NR	A, NA	Plasma	HPLC	0	315% and 74%* increase of A and NA 0 mins post in men 264% and 38% increase of A and NA 0 mins post in women
Raastad et al. (95)	Pre-post with control	9 (9/0)	26.9±1.4	Professional athletes	Back squats, front squats, leg extensions	3 x 3-6; 4-6	100% 3-6RM	08:30-10:00	ACTH C, GH	Plasma Serum	ILMA RIA, IRMA	0, 15, 30, 45, 60	43% and 24% decrease of ACTH and C 0 mins post, no significance reported 1462% increase of GH 0 mins post Gradual decrease during recovery
Rahimi et al. (97)	Pre-post with control	10 (10/0)	22±2	Experienced	Squat, bench press	4 x NR; 1, 1,5 or 2	85% 1RM	09:00-11:00	GH	Serum	ELISA	0, 30	208%*, 142%* and 133% increase of GH 0 mins post in 60, 90 and 120 secs rest protocols

Reference	Design	N (m/f)	Age (years) M±SD	Resistance training status	Exercises	Volume Sets x Reps; Rest (mins)	Intensity	Time of day	Hormone	Sample	Analysis methods	Follow-up measurement (mins)	Main outcome related findings
Rahimi et al. (96)	Pre-post with control	10 (10/0)	22±2	Experienced	Squat, bench press	4 x NR; 1, 1,5 or 2	85% 1RM	09:00-11:00	C	Serum	ELISA	0, 30	93%*, 63%* and 13% increase of C 0 mins post in 60, 90 and 120 secs rest protocols
Smilios et al. (108)	Pre-post with control	11 (11/0)	23±4	Experienced	Bench press, lat. pulldowns, squat, overhead press	2, 4 or 6 x 5; 3	80-88% 1RM	09:00 or 11:30	C, GH	Serum	LIA, IRMA	0, 15, 30	18%*, 18%* and 24%* decrease of C 0 mins post in 2,4 and 6 set group; stayed below baseline during recovery 141%*, 448%* and 383%* increase of GH 0 mins post in 2,4 and 6 set group; stayed below baseline during recovery; returned towards baseline 30 mins post
Tsai et al. (114)	Pre-post with control	20 (20/0)	22.4±2.4	NR	Bench press, biceps curls, triceps extensions, leg press, vertical butterflies, leg extensions	2 x 10; 1.5-2	80% 1RM	Morning	C, GH	Serum	ELISA, CLIA	5	13%* decrease of C 5 mins post; 1157%* increase of GH 5 mins post
Walker et al. (123)	Pre-post with control	13 (13/0)	28.4±3.7	Inexperienced	Leg press	15 x 1; 3 or 5 x 10; 2	100% 1RM or 80% 1RM	M: 15:45	C, GH	Serum	CLIA	0, 15, 30	Exercise with variable loads induced sig. increase of C and GH 0, 15 and 30 mins post in 80% intensity group; C didn't change sig. and GH increased sig. 0 mins post in 100% group; Exercise with constant load induced no sig. change of C or GH in 100% group; C and GH increased sig. 15 and 30 mins post in 80% group
Wells et al. (126)	Pre-post	10 (10/0)	24.7±3.4	Experienced	Back squats, bilateral leg press, bilateral hamstring curls, bilateral leg extensions, seated calf raises	4-6 x 3-5; 3	90% 1RM	Morning	C	Plasma	ELISA	0, 30, 60	21% increase of C 0 mins post; decrease below baseline during recovery
Zafeiridis et al. (128)	Pre-post with control	10 (10/0)	22.8±4.1	Experienced	Bench press, lat. pulldowns, squat, overhead press	4 x 5; 3	88% 1RM	09:00-11:30	C, GH	Serum	LIA, IRMA	0, 30	18% decrease of C 0 mins post, stayed below baseline during recovery 400%* increase of GH 0 mins post; returned towards baseline 30 mins post

A adrenaline; *BDNF* brain-derived neurotrophic factor; *C* cortisol; *CLIA* Chemiluminescence immunoassay; *ECLIA* Electrochemiluminescence immunoassay; *ELISA* Enzyme-linked immunosorbent assay; *f* female; *FIA* Fluorescence immunoassay; *GH* growth hormone; *HPLC* High-performance liquid chromatography; *ILMA* Immunoluminometric assay; *IRMA* Immunoradiometric assay; *LIA* Luminescence immunoassay; *m* male; *M* mean; *mins* minutes; *N* number of participants in the included intervention groups; *NA* noradrenaline; *NR* not reported; *Reps* repetitions; *RIA* radioimmunoassay; *RM* repetition maximum; * significantly different from baseline value at $p < 0.05$

Table 1. Subject and intervention characteristics and results of the included studies

key outcome variables were described clearly in all studies. The majority of the studies described the reliability of the key outcome measures, while no study detailed their internal validity. The results of the studies were discussed adequately, although only eight studies stated the clinical implications clearly. The exact quality assessment scores of all included article are presented in Table 2.

Acute effects on primary outcomes

There were no studies identified through the database search that investigated the acute effects of heavy resistance exercise on acetylcholine, VIP, CGRP, substance p, serotonin, NGF or GDNF. Consequently, results are presented for cortisol, ACTH, GH, adrenaline, noradrenaline and BDNF, assigned to the respective pathway of neuroendocrine-immune regulation they are associated with.

Sympathetic pathway

Changes in the circulating levels of adrenaline and noradrenaline in response to a heavy resistance exercise bout were measured by 6 studies, including a total of 9 intervention groups [31, 33, 39, 41, 63, 94].

Immediate effects

All included studies reported an immediate increase in the peripheral concentrations of adrenaline and noradrenaline after the termination of the resistance exercise session. All studies recruited resistance training experienced participants and only one study [63] included an additional intervention group of untrained participants. Still, differences in the magnitude of the changes became apparent, although there were no studies involved comparing variables like session volume, rest period or muscle mass involved.

The increase of adrenaline across the studies and intervention groups ranged from 68% to 315%, with a median (IQR) increase of 185% (73-262) (see Figure 2A). The most prominent increase was elicited by the resistance exercise bout employed by Pullinen et al. [94], despite the fact that it exhibited the lowest volume across the studies (8-10 total repetitions per session) using a single-joint knee extension exercise. On the contrary, another study using a protocol with an identical intensity in terms of the relative load used, repetitions in reserve and a similar volume reported the smallest increase among the studies investigating catecholamines [63]. The comparison of the immediate response of adrenaline showed that, although the absolute concentrations post-exercise were significantly greater for athletes compared to untrained individuals (no significance level reported), both groups changed to a similar degree, suggesting that there was no considerable influence of exercise experience on the acute adrenaline response [63].

The increase of noradrenaline across the studies and intervention groups ranged from 38 to 424%, with a median (IQR) increase of 113% (74-314) (see Figure 2B). The results of the studies indicated that protocols using only one set elicited the smallest increase across the studies, while those that used multiple sets of one exercise listed greater increases. In contrast to adrenaline, Kraemer et al. [63] showed that the increase of noradrenaline was significantly greater (no significance level reported) for athletes than for untrained subjects.

Studies comparing the catecholamine responses of men and women did not report any significant differences in the absolute levels of adrenaline or noradrenaline, neither before nor after the exercise bout between both sexes. Still, the relative changes from baseline to post-exercise suggest a trend towards slightly more pronounced catecholamine increases in men compared to women [31, 94]. Additionally, there is evidence from a study that took multiple blood samples before and during the exercise bouts that catecholamines increase in anticipation of the exercise stimulus and might already peak during the exercise protocol [33].

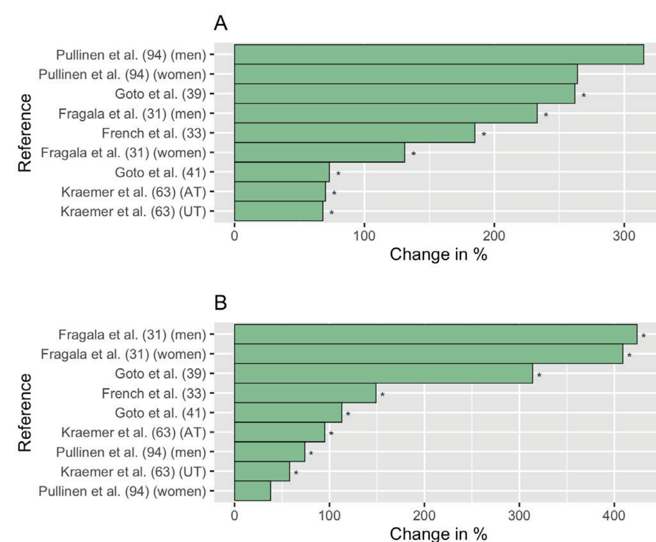

Figure 2. Immediate effects (0-5 minutes post-exercise) of heavy resistance exercise on **A** Adrenaline and **B** Noradrenaline expressed as change in %; * significantly different from baseline value at $p < 0.05$. AT=Professional athletes; UT=Untrained subjects

10-60 minutes post-exercise

Three of the six studies that investigated the catecholamine response to a resistance exercise session conducted follow-up measures during the recovery period [31, 39, 41]. None of the studies observed a significant difference in recovery values between the recovery period and their respective baselines. All of them reported that adrenaline and noradrenaline gradually decreased towards the baseline value at 15, 30 and 60 minutes into recovery.

Neuroendocrine pathway

A total of 26 studies with 46 intervention groups [6, 9, 19, 20, 23, 27, 39, 41, 45, 49, 52, 60–63, 69, 75, 82, 89, 95, 96, 108, 114, 123, 126, 128] and 18 studies with 34 intervention groups [39–41, 45, 60, 62, 64, 65, 69, 75, 82, 89, 95, 97, 108, 114, 123, 128] investigated the acute response of cortisol and GH respectively. One study also described the changes of ACTH in response to heavy resistance exercise [95].

Immediate effects

The results of the studies investigating the immediate effect of resistance exercise on the hormones of the neuroendocrine pathway revealed different response patterns for the different hormones in both the magnitude and the direction of the change. The peripheral concentration of ACTH was described to exhibit a decrease following a lower body resistance exercise bout in the morning [95]. The authors reported that the ACTH response appeared to be associated with the response of

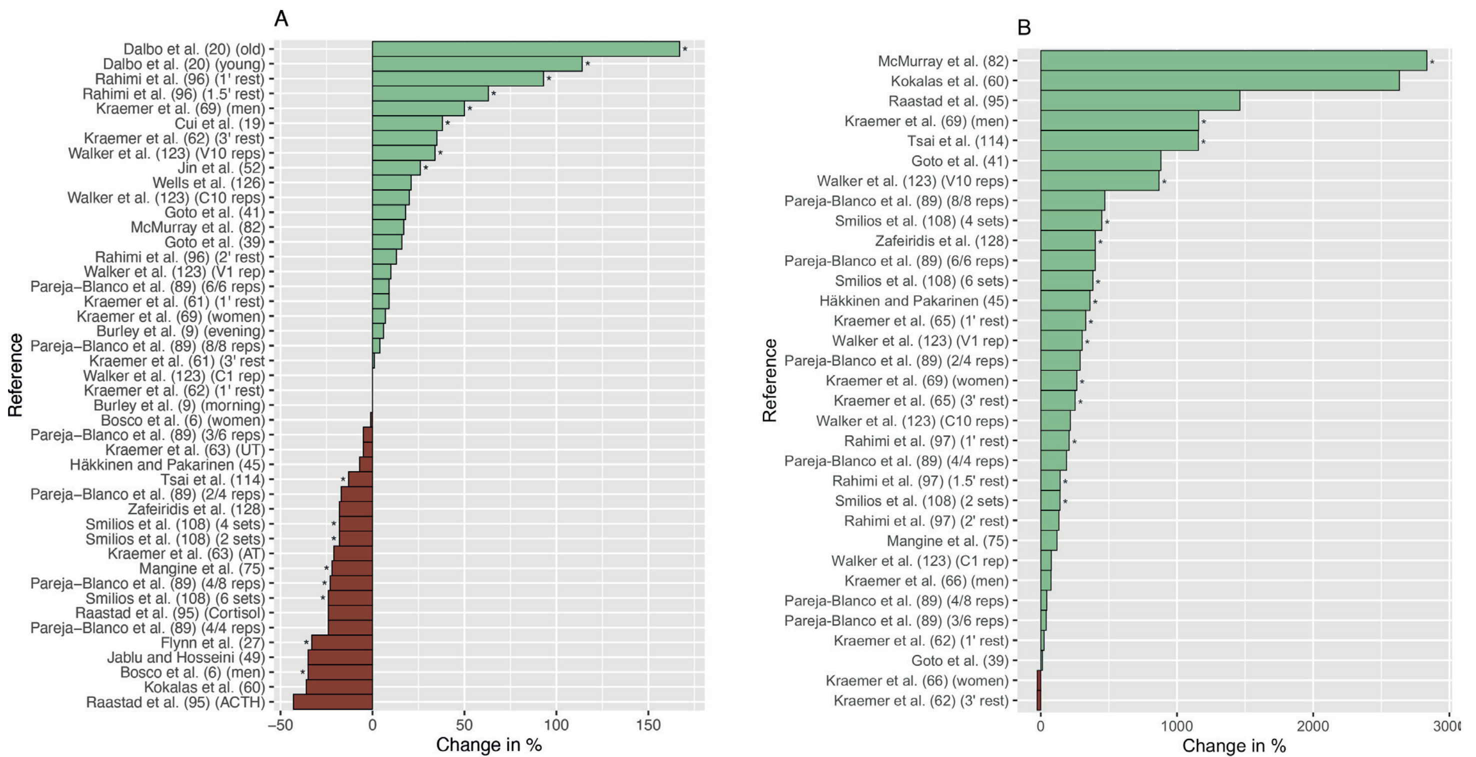

Figure 3. Immediate effects (0-5 minutes post-exercise) of heavy resistance exercise on **A** Cortisol and **B** Growth Hormone expressed as change in %; * significantly different from baseline value at $p < 0.05$. AT=Professional athletes; UT=Untrained subjects; C1/10 rep(s)=1/10 repetition(s) with constant resistance; V1/10 rep(s)=1/10 repetition(s) with variable resistance; 2/4 reps=half-maximal repetitions, 4 intended but 2 executed; 4/4=maximal repetitions, 4 executed

cortisol that decreased to a smaller amount [95].

Nonetheless, overall, the results regarding the direction of the change of cortisol following resistance exercise remained contradictory as they ranged from -48% to 167% across the included studied and intervention groups (see Figure 3A). The median (IQR) change of 0% (-18-18) shows that the participants in one half of the intervention groups exhibited an increase, while the other half exhibited a decrease in the circulating concentrations of cortisol immediately after the session. The exercise protocols of half of the intervention groups resulted in changes between -18% and 18%. The magnitude of the change appears to be associated with the sex of the investigated participants, with two studies reporting more pronounced responses in males compared to females [6, 69]. While males exhibited significant increases and decreases of cortisol immediately after exercise, the levels remained almost unchanged in females [6, 69]. A study by Dalbo and colleagues (2011) reported the biggest increase of cortisol following a lower body exercise bout performed by a group of young and a group of old men. They detected that the absolute peripheral cortisol concentrations were lower in older subjects at baseline and post-exercise. Nevertheless, the increase of cortisol was more pronounced in older subjects [20]. The influence of training status on cortisol alterations was only investigated in one study that reported no significant differences in the absolute levels between athletes and untrained subjects at baseline or follow-up but showed a slightly bigger decrease in athletes [63]. Studies investigating young male subjects with resistance training experience found that shorter inter-set rest intervals of one minute induced greater changes than two or three minutes of rest respectively [61, 96]. Yet, the opposite results were discovered with young resistance trained women when using the same exercise protocol, showing greater changes with longer rest periods [62]. Burley et al. [9] observed the circadian influence on the absolute peripheral cortisol concentrations as they showed significantly greater ($p < 0.001$) levels during a morning compared to an evening whole body exercise bout. The changes from baseline to post-exercise however did not seem to be influenced by the time of the day [9]. The comparison of 6 exercise protocols, of which three were performed with the maximum number of repetitions until fatigue was reached and three with half of the maximum number of repetitions revealed no clear evidence that the cortisol response is dependent on the number of repetitions in reserve or muscular fatigue [89]. It could however be shown that the two protocols with the highest session volume were the only two to induce cortisol increments post-exercise [89]. Similarly, an investigation comparing two exercise protocols involving variable resistance with two protocols involving constant resistance showed that in both protocols the bouts with the respective higher volume induced a greater cortisol increase [123]. Smilios et al. [108] on the other hand found no considerable differences between the changes induced by a 2-, 4- or 6-set protocol, as all of them decreased to similar amounts.

The change of growth hormone ranged from -27% to 2835% across studies and intervention groups, with a median (IQR) increase of 265% (119-448) (see Figure 3B). A comparison of the GH responses of men and women revealed that men tended to exhibit greater increments of GH immediately after a resistance exercise bout. The magnitude of these increases as well as the disparity between the responses of men and women were greater in a study including resistance trained

Criteria	Scoring	Bosco et al. (6)	Burley et al. (9)	Church et al. (13)	Cui et al. (19)	Dalbo et al. (20)	Doma et al. (23)	Flynn et al. (27)	Fragala et al. (31)	French et al. (33)	Goto et al. (40)	Goto et al. (41)	Goto et al. (39)	Häkkinen & Pakarinen (45)	Jablu & Hosseini (49)
1. Research aims or questions stated clearly	1 – Yes; 0.5 – yes, lacking detail or clarity; 0 – no	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2. Participants detailed	1–Yes; 0 – no														
Number		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Age		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sex		1	1	1	0	1	1	1	1	1	1	1	1	1	1
Height		1	0	1	0	0	1	1	1	1	1	1	1	1	1
Sub Total		1	0.75	1	0.5	0.75	1	1	1	1	1	1	1	1	1
3. Recruitment and sampling methods described	1 – Yes; 0.5 – yes, lacking detail or clarity; 0 – no	0.5	0	0	0.5	0	0	1	0	0.5	0.5	0	0.5	0.5	0.5
4. Inclusion and exclusion criteria detailed	1 – Yes; 0.5 – yes, lacking detail or clarity; 0 – no	0.5	0.5	0.5	1	1	0.5	1	0.5	0.5	0.5	0	0.5	0.5	1
5. Key outcome variables clearly described	1 – Yes; 0.5 – only some defined; 0.5 – yes, lacking detail or clarity; 0 – no	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6. Adequate methodology able to repeat study	1 – Yes; 0 – no														
Participant sampling		0	0	0	0	0	0	1	0	1	1	0	0	1	1
Equipment		1	1	1	1	1	1	0	1	1	1	1	1	1	1
Procedure		1	1	0	0	1	0	0	1	1	0	1	1	1	1
Data processing		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Statistical analysis		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sub total		0.8	0.8	0.6	0.6	0.8	0.6	0.6	0.8	1	0.8	0.8	0.8	1	1
7. Methodology able to answer research question	1 – Yes; 0 – no														
Participant sampling		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Equipment		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Procedure		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Data processing		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Statistical analysis		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sub total		1	1	1	1	1	1	1	1	1	1	1	1	1	1
8. Reliability of the methodology stated	1–Yes; 0–no	1	1	1	0	1	1	1	0	1	0	0	1	1	0
9. Internal validity of the methodology stated	1–Yes; 0–no	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10. Research questions answered adequately in the discussion	1–Yes; 0–no	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11. Key findings supported by the results	1–Yes; 0–no	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12. Key findings interpreted in a logical manner which is supported by reference	1–Yes; 0–no	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13. Clinical implications stated	1 – Yes; 0.5 – yes, lacking detail or clarity; 0 – no	0.5	0.5	0	0.5	1	1	1	1	1	1	1	1	1	1

Criteria	Scoring	Jin et al. (52)	Kokalas et al. (60)	Kraemer et al. (65)	Kraemer et al. (66)	Kraemer et al. (62)	Kraemer et al. (61)	Kraemer et al. (69)	Kraemer et al. (63)	Mangine et al. (75)	Marston et al. (77)	Marston et al. (76)	McMurray et al. (82)	Pareja-Blanco et al. (89)	Pullinen et al. (94)
1. Research aims or questions stated clearly	1 – Yes; 0.5 – yes, lacking detail or clarity; 0 – no	0.5	1	1	1	1	1	1	1	1	1	1	0.5	1	1
2. Participants detailed	1–Yes; 0 – no														
Number		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Age		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Sex		1	1	1	1	1	1	1	1	1	1	0	1	1	1
Height		1	1	1	1	1	1	1	1	1	0	1	1	1	1
Sub Total		1	1	1	1	1	1	1	1	1	0.75	0.75	1	1	1
3. Recruitment and sampling methods described	1 – Yes; 0.5 – yes, lacking detail or clarity; 0 – no	0	1	0	0	0.5	0	0.5	0	0.5	0	0.5	0	0.5	0
4. Inclusion and exclusion criteria detailed	1 – Yes; 0.5 – yes, lacking detail or clarity; 0 – no	0.5	0.5	0.5	0.5	1	0.5	0	0.5	0.5	0.5	0.5	0.5	1	0
5. Key outcome variables clearly described	1 – Yes; 0.5 – only some defined; 0.5 – yes, lacking detail or clarity; 0 – no	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6. Adequate methodology able to repeat study	1 – Yes; 0 – no														
Participant sampling		0	1	1	1	1	1	1	1	1	0	0	0	1	0
Equipment		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Procedure		0	1	0	1	1	0	0	0	0	0	0	1	0	0
Data processing		0	1	1	1	1	1	1	1	1	1	1	1	1	1
Statistical analysis		1	1	0	0	1	0	0	1	1	1	1	1	1	1
Sub total		0.4	1	0.6	0.8	1	0.6	0.6	0.8	0.8	0.6	0.6	0.8	0.8	0.6
7. Methodology able to answer research question	1 – Yes; 0 – no														
Participant sampling		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Equipment		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Procedure		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Data processing		1	1	1	1	1	1	1	1	1	1	1	1	1	1
Statistical analysis		0	1	1	1	0	1	1	1	1	1	1	1	1	1
Sub total		0.8	1	1	1	0.8	1	1	1	1	1	1	1	1	1
8. Reliability of the methodology stated	1–Yes; 0–no	0	1	1	1	1	1	1	1	1	1	1	0	0	0
9. Internal validity of the methodology stated	1–Yes; 0–no	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10. Research questions answered adequately in the discussion	1–Yes; 0–no	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11. Key findings supported by the results	1–Yes; 0–no	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12. Key findings interpreted in a logical manner which is supported by reference	1–Yes; 0–no	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13. Clinical implications stated	1 – Yes; 0.5 – yes, lacking detail or clarity; 0 – no	0.5	1	0.5	0	0	0.5	0.5	0	1	0.5	1	0	1	0

Table 2. Methodological quality of the included studies

Criteria	Scoring	Raastad et al. (95)	Rahimi et al. (97)	Rahimi et al. (96)	Smilios et al. (108)	Tsai et al. (114)	Walker et al. (123)	Wells et al. (126)	Zafeiridis et al. (128)
1. Research aims or questions stated clearly	1 – Yes; 0.5 – yes, lacking detail or clarity; 0 – no	1	1	1	1	1	1	1	1
2. Participants detailed	1–Yes; 0 – no								
Number		1	1	1	1	1	1	1	1
Age		1	1	1	1	1	1	1	1
Sex		1	1	1	1	1	1	1	1
Height		0	0	1	1	0	1	1	1
Sub Total		0.75	0.75	1	1	0.75	1	1	1
3. Recruitment and sampling methods described	1 – Yes; 0.5 – yes, lacking detail or clarity; 0 – no	0.5	0	0	0	1	0	1	0
4. Inclusion and exclusion criteria detailed	1 – Yes; 0.5 – yes, lacking detail or clarity; 0 – no	0	0.5	0.5	0	1	0	1	0
5. Key outcome variables clearly described	1 – Yes; 0.5 – only some defined; 0.5 – yes, lacking detail or clarity; 0 – no	1	1	1	1	1	1	1	1
6. Adequate methodology able to repeat study	1 – Yes; 0 – no								
Participant sampling		0	1	1	0	1	0	1	0
Equipment		1	1	1	1	1	1	1	1
Procedure		1	0	0	1	1	0	1	1
Data processing		1	1	1	1	1	1	1	1
Statistical analysis		1	0	1	1	1	1	1	1
Sub total		0.8	0.6	0.8	0.8	1	0.6	1	0.8
7. Methodology able to answer research question	1 – Yes; 0 – no								
Participant sampling		1	1	1	1	1	1	1	1
Equipment		1	1	1	1	1	1	1	1
Procedure		1	1	1	1	1	1	1	1
Data processing		1	1	1	1	1	1	1	1
Statistical analysis		1	1	1	1	1	1	1	1
Sub total		1	1	1	1	1	1	1	1
8. Reliability of the methodology stated	1–Yes; 0–no	1	1	1	1	0	1	1	1
9. Internal validity of the methodology stated	1–Yes; 0–no	0	0	0	0	0	0	0	0
10. Research questions answered adequately in the discussion	1–Yes; 0–no	1	1	1	1	1	1	1	1
11. Key findings supported by the results	1–Yes; 0–no	1	1	1	1	1	1	1	1
12. Key findings interpreted in a logical manner which is supported by reference	1–Yes; 0–no	1	1	1	1	1	1	1	1
13. Clinical implications stated	1 – Yes; 0.5 – yes, lacking detail or clarity; 0 – no	0	1	0.5	0.5	0.5	0.5	0.5	0

inexperienced participants compared to those who already had some experience with resistance training [64, 69]. Across the included studies, the intervention groups investigating female subjects reported a median change of GH concentrations of -1%, while the intervention groups investigating male subjects reported a median increase of 304%. The findings regarding the potential influence of exercise parameters on the GH response suggest that the rest intervals between the sets influences the magnitude of the acute response to exercise. Two studies [62, 65] employing the same exercise protocol reported that the shorter rest interval of one minute induced greater increases in peripheral GH concentration than a three-minute rest interval [65]. This is supported by another study that described significant increases of GH in a one-minute rest interval protocol but no significant change in a two-minute protocol [97]. Another training variable that appears to influence the magnitude of the GH response is the number of repetitions that are left in reserve during a set. A study including ten young resistance trained males described greater increase of GH for sets that were performed to failure compared to those that required only the half-maximal number of repetitions [89]. Additionally, those sets that were performed with a higher number of repetitions and thus with a higher session volume led to greater increases of GH [89, 108, 123].

10-60 minutes post-exercise

13 studies offered insight into the recovery of cortisol concentrations following the immediate response to heavy resistance exercise. The early recovery period of cortisol between 10 and 30 minutes after the termination of the exercise bout was characterized by an unstable trajectory, without a clear perceptible pattern. Four studies [62, 95, 96, 108] reported an intervention group that exhibited a further gradual increase or decrease of cortisol concentrations in the early recovery period. Five studies [39, 41, 49, 61, 126] reported that in at least one of the investigated intervention groups the trajectories of the cortisol response changed and either decreased below the baseline value or changed towards the baseline value. In four studies [52, 75, 82, 128] the cortisol levels remained approximately at the same levels as immediately after the termination of the exercise session. 60 minutes after the termination of the exercise however, these studies reported a decrease of cortisol below the baseline value [75, 82]. This trend was also observed by all other studies that took follow-up measures at 60 minutes into recovery [45, 95, 126], except for one [19].

Although sex, rest intervals and the number of repetitions appeared to influence the immediate GH response there was no clear evidence that indicated an influence of these parameters on the recovery of GH levels post-exercise. While five studies [39, 41, 64, 97, 123] reported that GH increased further in at least one of the investigated intervention groups during the early recovery period (10-30 minutes), the majority of the studies that employed multiple follow-up measures described a decline of the GH levels 10-30 minutes into recovery. A study investigating 13 male participants before, 15 minutes and 30 minutes after leg press exercise reported that the GH concentrations further increased in the higher volume groups with a peak at 15 minutes, while they gradually decreased in the lower volume groups [123]. All studies that employed follow-up measures 60 minutes post-exercise described that peripheral GH decreased towards - or in most cases even below - the resting value [40, 45, 62, 64, 65, 75, 82, 95].

Neurotrophic pathway

Neurotrophic biomarkers were measured by three of the included studies in the form of BDNF [13, 76, 77], while there were no studies identified that examined NGF or GDNF.

Immediate effects

The general magnitude and direction of the change of BDNF in the circulation elicited by resistance exercise varied across the three studies with one reporting a significant increase [13] and two describing small changes in a negative [77] and positive direction [76]. The most prominent immediate change was detected by Church et al. [13]. In a study investigating ten young male subjects with resistance training experience blood was sampled before and during recovery after the very first resistance exercise session of an 8-week training program. The training session involving multiple muscle groups induced a statistically significant increase in peripheral BDNF concentrations of 63% ($p < 0.05$). Two studies conducted by Marston et al. [77] and Marston et al. [76] on the other hand were not able to detect any considerable changes of peripheral BDNF concentrations immediately after resistance exercise. In both investigations, the authors recruited subjects of both sexes without resistance training experience. The study including late-middle-aged adults reported a BDNF increase of 3% immediately after termination of the exercise session [76] (see Figure 4). BDNF levels of young adults showed a small decrease of -1%, even though they trained with a higher volume in an otherwise comparable protocol [77].

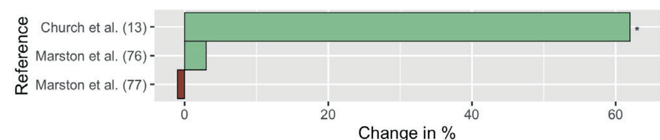

Figure 4. Immediate effects (0-5 minutes post-exercise) of heavy resistance exercise on Brain derived neurotrophic factor expressed as change in %; * significantly different from baseline value at $p < 0.05$.

10-60 minutes post-exercise

All three studies took follow-up measures during the acute recovery period. The trajectories of the biomarker concentrations during recovery mimicked the acute response in all studies, showing only small deviations from the immediate response. 30 minutes into recovery the circulating levels of BDNF decreased in both studies by Marston and colleagues slightly below the baseline and immediate post-exercise values. Similarly, Church et al. [13] reported a decrease of BDNF from 0 to 30 minutes post-exercise, even though it stayed statistically significantly elevated compared to baseline. 60 minutes into recovery however the levels increased again.

DISCUSSION

The aim of the present review was to examine the acute effects of heavy resistance exercise on selected biomarkers of neuroendocrine-immune regulation. These effects were defined as the change in the circulating concentration from a baseline resting level to a post-exercise level. To the authors'

knowledge this is the first systematic review to examine the acute response of these biomarkers to this specific type of exercise stimulus.

The findings of this systematic review revealed that the effects elicited by a heavy resistance exercise bout vary between the investigated biomarkers (see Figure 5). finding of a general acute increase of catecholamine levels in response to heavy resistance exercise is in line with results of studies that characterized a release of catecholamines not only in response to physical stress like aerobic exercise [66] or moderate intensity resistance exercise [10, 43] but also as a result of cognitive stress [12].

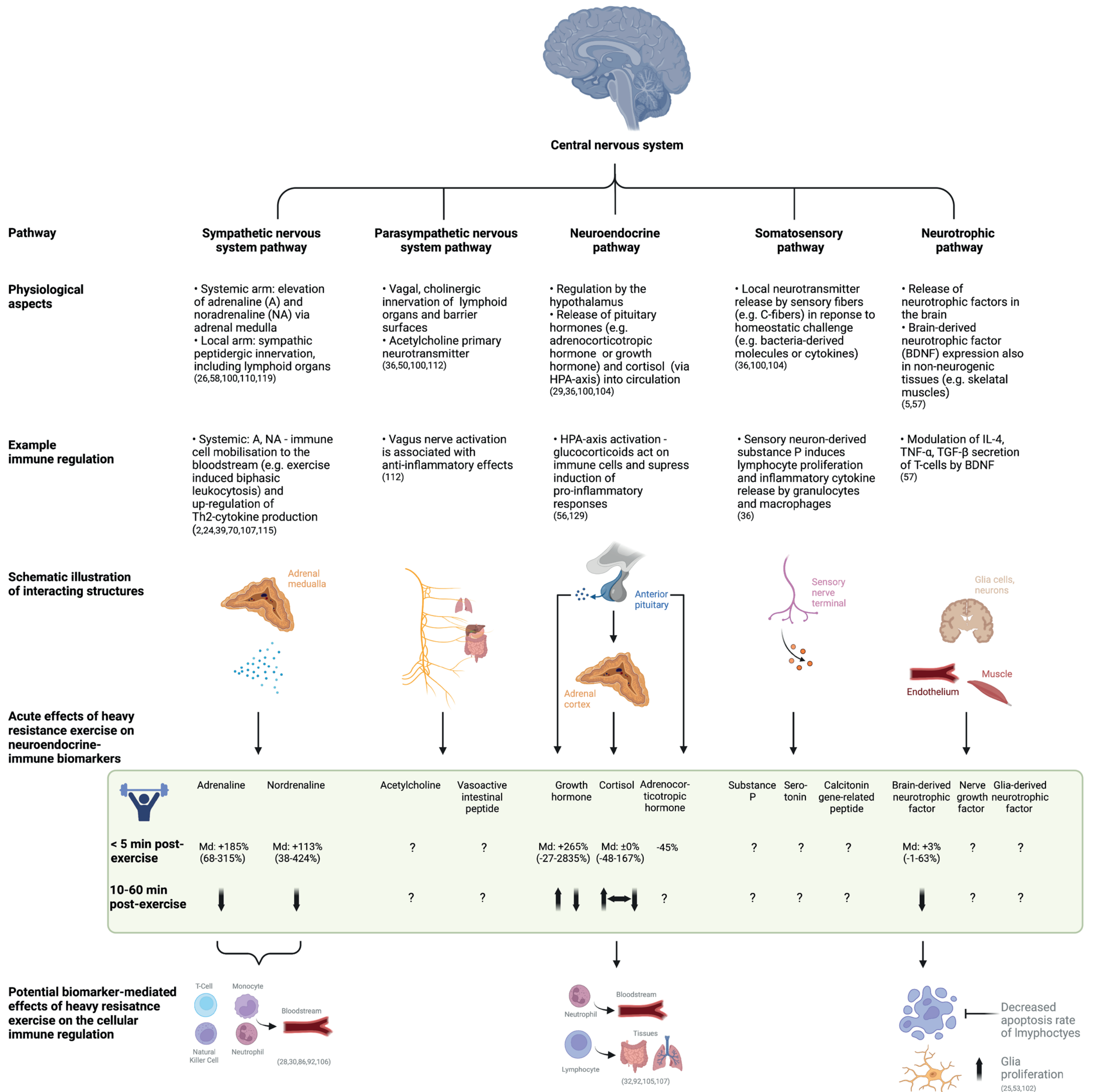

Figure 5. Investigated pathways of neuroendocrine-immune regulation, acute changes of associated biomarker concentrations following a bout of heavy resistance exercise and potential effects on the cellular immune regulation. The upwards arrow (🡅) indicates a further increase, the downwards arrow (🡇) a further decrease and the left-right arrow (⟺) no further changes of biomarker concentrations during recovery from a bout of heavy resistance exercise. HPA-axis=hypothalamic-pituitary-adrenal axis; Md=median (Graphic created with BioRender.com)

Even though the included studies employed different exercise protocols, adrenaline and noradrenaline were uniformly found to increase immediately after the termination of the exercise session and to gradually return to baseline during the first hour of recovery [31, 33, 39, 41, 63, 94]. The

This increased sympathetic signaling has previously been described as the organism's first response to a stressful stimulus to prepare and enable the body to cope with the stressful situation in the context of a fight-or-flight reaction [88]. Correspondingly, results provided in studies in the present

review suggest that peripheral catecholamine concentrations in¬ response to exercise already peak during the exercise period [33] and that the body releases catecholamines in anticipation of the stressor to be prepared once the resource demand increases [33, 63]. As the influence of individual training status and the sex of the participants has only been investigated by one [63] and two studies [31, 94], respectively, we cannot draw any firm conclusion on whether both parameters influence the acute sympathetic response. Still, the results of the studies give an indication that men, as well as athletes might experience greater increases of catecholamine levels in response to heavy resistance training compared to women and untrained individuals, respectively.

Systemically, the increased concentration of adrenaline and noradrenaline induces an increased heart rate, blood pressure and serum glucose levels as well as a bronchodilation [88]. However, it bears noting that noradrenaline is predominantly released by nerve terminals and should therefore be considered a neurotransmitter rather than a hormone [38]. Hence, plasma levels of noradrenaline are not always a reliable measure for sympathetic activity, as it exerts its effects rather locally than systemically [99]. Locally, it controls the vascular diameter and directs the peripheral blood distribution during daily activities, including exercise [38]. The higher increments of noradrenaline, but not of adrenaline, reported by Kraemer et al. [63] could therefore indicate that the sympathetic activity of athletes is characterized by a similar adrenal output, but greater noradrenaline release of sympathetic nerve terminals compared to untrained individuals. Irrespective of whether catecholamines are released via the adrenal medulla (80% adrenaline) or via postganglionic sympathetic nerve fibers in approximation with lymphoid target tissues like bone marrow, lymph nodes or mucosal barriers (mainly noradrenaline), their effects on the immune system are primarily mediated by β2-adrenergic receptors expressed by leukocytes [73, 88]. The stimulation of adrenergic receptors impacts immune cells with regard to their function, proliferation and trafficking [36, 119]. The most commonly reported effect of β-adrenergic signaling on the immune system is the mobilization and redistribution of leukocytes into the circulation [30, 92, 117]. An adrenaline-induced mobilization of NK-cells in response to running exercise in mice was for example described by Pedersen et al. [93]. On the contrary, the blockade of β-adrenergic receptors resulted in no leukocytosis after exercise [2]. The likelihood of any receptor interactions might be, at least for some leukocyte subpopulations, further facilitated by heavy resistance exercise. Fragala et al. [31] reported that the expression of β2-adrenergic receptors on lymphocytes was elevated after the termination of a heavy resistance exercise bout. The expression on monocytes was increased in anticipation of the exercise bout but decreased during the exercise period [31]. Furthermore, enhanced activation of postganglionic sympathetic nerve fibers in response to exercise could affect the lymphocyte function and proliferation in lymphoid organs like the thymus, spleen and lymph nodes. Accompanied by a transient noradrenaline release, a higher sympathetic activity has been reported to promote a Th2-cell cytokine profile [24, 36].

Similar to the acute catecholamine response, increments in GH concentration immediately after exercise were reported by all studies, except for two (see Figure 3B). It has also been a consistent finding among the studies that employed follow-up measures that the elevated levels of GH returned towards their resting values within the first hour of recovery, albeit only two studies detailed the design of the post-exercise period. The maximal increases however were bigger and the range of the effects among the studies broader, compared to adrenaline and noradrenaline. Acute increases of GH after resistance exercise have previously been described by many other studies employing moderate load resistance training [17, 42, 118]. Still, it should be mentioned that GH cannot be considered as a single substance but rather as a family of proteins with several isoforms and molecular weights [4, 68]. Studies investigating the trajectory of different GH isoforms in response to cycling [124] or resistance exercise [47] reported increases of almost all isoforms post-exercise. The relationships of the different isoforms did however change, as some increased more than others. It has been described that routine immunoassays will only analyze a specific spectrum of total GH isoforms, depending on their specificity [46, 103]. The isoform specificity can vary between immunoassay principle and between manufactures. A characterization is possible but rarely conducted in practice, a circumstance that may affect the comparability of the results [103]. The studies included in this systematic review did not differentiate between different isoforms and only two studies [75, 123] specified that they analyzed the isoform with a molecular mass of 22kDa. Thus, it cannot be determined with certainty which isoforms were respectively measured.

Evidence has been found that the aforementioned changes of GH levels post-exercise are in part influenced by participant characteristics and intervention parameters. Two studies included in the present review detected greater GH increments in men compared to women [64, 69]. According to earlier reviews, this sex difference is uniform across different exercise types and might be attributable to a greater growth hormone mass per burst and higher sensitivity of GH to GH-releasing hormone in women compared to men [18, 120]. Furthermore, it has been reported that the GH response to heavy resistance exercise tended to be more pronounced when the rest intervals between the sets were shorter [62, 65, 97] and the session volume higher [89, 108, 123]. These findings support the notion that volume and intensity are factors that determine the GH release. Elevations in the circulating levels of GH promote anabolic processes. It increases protein synthesis and reduces the breakdown of muscle protein [83]. Likewise, GH plays an important role for the development of lymphoid organs and the proliferation of T-cells. These effects are exerted directly via GH-receptor signaling, but also through up-regulation of other receptor types like androgen or angiotensin II-receptors [73]. Still, the acute contributions of GH to the immunoregulation in response to exercise are not well understood. It is assumed that GH does not play an important role in the mobilization of lymphocytes following exercise. Instead, it could act in concert with adrenaline and noradrenaline to recruit neutrophils into the circulation [92]. This evidence however comes from studies that administered GH intravenously or inhibited progenitors of GH during stressful events [92].

In contrast to the previously discussed biomarkers, the effects on cortisol were characterized by a variation in the direction of the change from baseline to immediately post-exercise. Out of all biomarkers investigated in this systematic review, cortisol was the most frequently measured. At the same

time, the results regarding the direction and magnitude of the immediate response to heavy resistance exercise are the most ambiguous. One half of the intervention groups investigated in the included studies exhibited an increase or no change of cortisol post-exercise, while the other half responded with no change or a decrease of cortisol (see Figure 3A). Previous reviews have reported that hypertrophy-based schemes with moderate loads uniformly induced increases of circulating cortisol levels that were on average greater than the alterations induced by strength-based schemes with heavy loads [18, 67]. Protocols that aim to increase muscle mass rather than strength usually use more repetitions, thus higher session volumes and shorter rest intervals. Consequently, they are considered to be more stressful and metabolically demanding, an assumption that is supported by the fact that hypertrophy-based protocols elicited higher lactate responses that are positively correlated with cortisol concentrations [67]. The release of the catabolic glucocorticoid into the circulation is meant to help the organism to cope with the stressful situation, for example by breaking down protein to provide glucose or by antagonizing the protein synthesis [88]. It is conceivable that some exercise protocols were not intense enough to force the body to mobilize resources through the activation of the hypothalamic-pituitary-adrenal axis. Correspondingly, based on the included studies, it can be assumed that the cortisol responses tend to be greater in protocols with shorter rest periods, higher volumes and with sets performed until volitional muscular fatigue was reached. Nevertheless, this trend can neither be confirmed nor ruled out with certainty, since among the concerning studies, two did not support this notion [62, 108].

In addition to this hypothesis, it is conceivable that the existence of cortisol-responder and non-responder participant characteristics contributed to the ambiguous results regarding the direction of the effect of exercise among the included studies. A study investigating 21 young male subjects for instance described two patterns of cortisol responses to a one-hour cycling bout [105]. 13 participants exhibited increased cortisol levels post-exercise, while eight subjects did not show any increments in cortisol levels, even though there were no differences in terms of age, physical build, aerobic fitness, relative work rate or catecholamine response between the groups [105]. Based on this, this study also provided insights into the effects of cortisol on the immune system. Both groups exhibited an increase of granulocytes after the exercise bout, whereas the lymphocyte counts of cortisol non-responders returned to baseline and the cortisol responders exhibited a significant lymphopenia [105]. Further it has been described that cortisol induces and maintains a neutrophilia some hours after release or administration [32, 92, 107], by binding intracellular, ligand-gated glucocorticoid receptors, expressed by virtually all nucleated cells in the human organism, including leukocytes [14, 22, 111]. Equally important is the control of pro-inflammatory cytokines and the stimulation of regulatory T-cell activity by cortisol [56, 129]. ACTH does not only stimulate the release of cortisol from the adrenal cortex but also exerts diverse effects on the immune system. The binding of ACTH to leukocytic receptors has the potential to inhibit certain immunological processes, such as the production of antibodies or interferons [54]. Owing to the fact that only one study included in the present review investigated the response of ACTH to heavy resistance exercise, its post-exercise kinetics remain unclear. From a physiological point of view, it can be assumed that ACTH reflects the activation of the hypothalamic-pituitary-adrenal axis in the wake of stressful stimuli.

Given the relatively small number of studies investigating the acute effects of exercise on BDNF, no firm conclusions can be drawn on the exercise-induced changes of the peripheral levels of BDNF. Several publications have previously reported significant releases of BDNF following both resistance [127] and aerobic exercise [59] as it has also been shown by Church et al. [13] and to a small degree also by Marston et al. [76] following a heavy resistance exercise bout. Nonetheless, the effects of resistance exercise on the BDNF levels in the circulation remain controversial, since some studies were not able to detect acute increases post-exercise [16, 26, 37]. Marston et al. [77] are therefore suggesting to consider the rest intervals, session volume and the blood samples used to quantify BDNF levels. None of the studies included in this systematic review compared the mentioned training parameters using heavy loading protocols. However, when comparing the heavy resistance exercise protocols employed in both studies by Marston et al. [76, 77], the one with the lower session volume (100 total repetitions per session) elicited a change of +3% [123] while serum BDNF decreased in the higher volume protocol (175 total repetitions per session) by 1% [77]. Still, it is advised to be cautious when comparing results of different studies given the different measurement contexts. From the discussed studies, it cannot be inferred that heavy resistance exercise bout-induced changes of BDNF are subject to a specific response pattern. Additionally, it can neither be confirmed nor ruled out that individual subject characteristics or training parameter influence the acute response to heavy resistance exercise.

As a member of the neurotrophin family, BDNF is described to have neuroprotective effects and to enhance neuroplasticity [78]. In this role it contributes largely to the exercise-induced improvements in cognitive domains. Beyond that, BDNF engages in immunoregulatory processes by binding the tyrosine kinase B (TrkB) and p75 neurotrophin receptors (NTR) expressed by immune cells [5, 125]. Evidence accumulates that upon binding, BDNF serves as an anti-apoptotic survival factor for B- and T-cells [125]. It was for example demonstrated that the B-cell development in the bone marrow is impaired in BDNF deficient mice, resulting in reduced number of B-cells in the peripheral blood [102] and that blocking BDNF through monoclonal antibodies increased the apoptosis rate of B-cells in vitro [25]. Additionally, it has been reported that BDNF alters the expression of cytokine mRNA in T-cells, modulating the secretion of IL-4, TNF-α and TGF-β [57]. Furthermore, by promoting the proliferation of glial cells, BDNF affects the first line of the cellular immune defense in the central nervous system [53]. Although the influence of BDNF on immune cells is now widely acknowledged, its role in the immunoregulation after exercise remains elusive. The fact that endurance exercise stress at maximal exertion but not at a moderate intensity upregulated the expression of p75 NTR on peripheral blood mononuclear cells (PBMC) could indicate that a certain intensity threshold needs to be reached in order to convey the reported effects in the context of exercise [7].

Similar to other reviews, there were no studies identified through the systematic literature search that investigated the

acute effects of heavy resistance exercise on acetylcholine, VIP, CGRP, substance p, serotonin, NGF or GDNF. The biomarkers discussed in this review were likely investigated because of their well-recognized involvement in the anabolic and catabolic adaptions following resistance exercise. Especially, biomarkers associated with parasympathetic and sensory nervous pathways might however be of less interest in strength and conditioning research, because they do not drive major muscular adaptions. Furthermore, their blood concentrations might be below the level that allows the detection of clinically relevant changes with conventional sampling methods [55].

Combining the findings of the present review and the discussed evidence regarding the effects of the selected biomarkers on the immune system in the context of exercise, it can be summarized that resistance exercise leads to transient changes in the activity of some pathways, whereby a divergent humoral milieu is created. Consequently, changes in immune system function that have been discussed for each biomarker are likely to occur. However, since the biomarkers are part of an orchestrated stress response, their impact on the immune system should not only be seen in an isolated way.

The general immunological adaptions to exercise have been documented many times during and following endurance exercise. The first response of the cellular department is characterized by a rapid mobilization of leukocytes into the blood stream (leukocytosis) [87]. Leukocyte counts, especially of neutrophils and lymphocytes, are reported to increase up to fivefold after endurance exercise stress [107]. The extent to which lymphocyte subsets are mobilized is dependent on the differential expression of adrenergic receptors on the cell surface, which underpins the significance of adrenaline and noradrenaline in the initial immune response. Accordingly, lymphocytes with an increased cytotoxic effector function such as NK-cells and CD8+ T-cells are preferentially redeployed [11, 107]. Conversely, subtypes in an early maturation stage or with limited cytotoxicity such as B-cells and CD4+ T-cells are less frequently mobilized [107]. Besides that, the magnitude of this response is dependent on exercise program variables such as intensity and duration. High intensity interval training or cycling sprints have been documented to cause greater cellular immune responses than continuous endurance exercise [51, 121]. Likewise, when intensities are matched, exercise bouts of longer duration cause greater leukocyte increases [80]. During the first hour of recovery, a fast reduction of circulating lymphocytes below the baseline has been documented, while neutrophil counts remain elevated [107], a phenomenon that is attributed to the time-lagged release of cortisol into the bloodstream [32, 92, 107]. Besides the adaptions of the cellular immune department, acute endurance exercise causes transient increases of circulating pro- (IL-1β, IL-6, TNF-α) and anti-inflammatory (IL-1ra, IL-10) cytokines [84, 116].

Even though the immunological adaptions to resistance exercise are not as extensively documented, there is evidence accumulating that they show a similar pattern to that observable following endurance exercise. It is a consistent finding across studies investigating the acute immunological response to resistance exercise in young to middle-aged subjects that total leukocyte counts increase immediately after the termination of the bout [8, 28, 86, 98, 106, 109]. Simonson et al. [106] for example demonstrated a biphasic immune regulation in response to a session of 8 x 8-10 repetitions at an intensity of 75% 1RM. The total leukocyte count and all measured subpopulations, except for basophils and eosinophils, increased following exercise, with NK-cells demonstrating the greatest increments. Subsequently, only neutrophils did not return to baseline levels by 30 minutes post-exercise [106]. In line with these findings, it was additionally reported that leukocytes, neutrophils and monocytes reached their maximum circulating levels two hours after the termination of the exercise bout [98] and that the levels of leukocytes and monocytes were still reduced 24 hours post-exercise [8].

Beyond that, the literature suggests that the acute immunological regulation in response to resistance exercise is impacted by program variables. Ihalainen et al. [48] demonstrated that the exercise stimulus must be of a certain length to cause immunological adaptions. Accordingly, leukocytosis occurred delayed following 15 sets of one repetition (MAX) compared to 5 sets of 10 repetitions (HYP) of leg press and lymphocytes did not increase at all following MAX [48]. Likewise, it was reported that a one-minute inter-set rest interval causes a significantly greater leukocytosis than a three-minute rest at the same total work [79].

It is however uncertain if, next to the general pattern and direction of the immunological adaptions to resistance exercise, the magnitude of these effects is also comparable to endurance exercise. To the author's knowledge only two studies have investigated this question and compared both exercise modes directly. Subjects that participated in a study comparing the cellular immune response to either 50 minutes of cycling or resistance exercise exhibited a leukocytosis, lymphocytosis and neutrophilia following both protocols. Yet, the alterations were greater in response to the endurance exercise bout for all immune cell subpopulations investigated [101]. These results were in line with a previously conducted study displaying a comparable experimental design [85].

Finally, considering the frequently stressed importance of catecholamines in the mobilization of immune cells and the consistently reported increase of catecholamines across studies in the present review, it is reasonable to assume that the initial immune response following a bout a heavy resistance exercise is characterized by a redistribution of leukocytes from storage sites. The release of cortisol in response to resistance exercise has previously been associated with a lymphocytopenia and maintained neutrophilia during recovery. In particular, a negative correlation of cortisol with T-helper cell counts has been reported 30 and 60 minutes after a submaximal resistance exercise session [98]. Reflecting the inconsistent results of the studies included in the present review regarding cortisol, it must be assumed that its effects on the immune system are only transmitted once an intensity threshold is reached and maintained for a sufficient period of time. Consequently, the redistribution of immune cells and their maintenance in the circulation appears to be significantly influenced by the ratio of cortisol and catecholamines [32]. The specific immunoregulatory effects that BDNF exerts in the context of exercise remain to be determined. The upregulation of BDNF-specific receptors after exercise on PBMC [7] however gives a strong indication that BDNF might not only be associated with enhanced neuroplasticity and neuroprotection post-exercise but also with "immunoprotection".

CONCLUSIONS

The present review showed that a bout of heavy resistance exercise alters the activity of specific pathways of neuroendocrine-immune regulation. Specifically, it leads to considerable increases in the peripheral concentrations of adrenaline, noradrenaline and GH immediately after the termination of the exercise bout. The reported changes in cortisol levels showed less homogeneous results and appear to be more sensitive to the configuration of the exercise scheme or individual subject characteristics.

The limited number of studies and their mixed results allow no firm conclusions to be drawn about the direction of the effect of heavy resistance exercise on the circulating BDNF and ACTH levels.

The duration of the rest periods between the sets seems to be the most influential factor for the magnitude of the response of cortisol and GH, as shorter rest periods tended to elicit greater changes. Men lean towards greater catecholamines increases than women.

Overall, the investigated biomarkers tended to return to baseline one hour after the termination of the exercise bout, albeit the recovery of cortisol showed no clear pattern. Notably, catecholamine levels exhibited the fastest recovery.

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Conflict of interest

The authors declare that there is no conflict of interest associated with the publication of this systematic review.

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Immunometabolism-fit: How exercise and training can modify T cell and macrophage metabolism in health and disease

José Cesar Rosa-Neto[1*], Fábio Santos Lira[2], Jonathan Peter Little[4], Graham Landells[4], Hashim Islam[4], Bénédicte Chazaud[5], David B. Pyne[6], Ana Maria Teixeira[7], Helena Batatinha[1], Barbara Moura Antunes[2,9], Luciele Guerra Minuzzi[2,7], Jana Palmowksi[8], Richard J Simpson[3], Karsten Krüger[8].

1. Department of Cell Biology and Development, Institute of Biomedical Sciences, University of São Paulo (USP), São Paulo, SP 05508-000, Brazil.
2. Exercise and Immunometabolism Research Group, Department of Physical Education, São Paulo State University (UNESP), Presidente Prudente 19060-900, Brazil.
3. School of Nutritional Sciences and Wellness; Department of Pediatrics; Department of Immunobiology, The University of Arizona, Tucson, AZ, USA.
4. School of Health and Exercise Sciences, University of British Columbia, Okanagan Campus, Kelowna, British Columbia, CANADA.
5. Institut NeuroMyoGene, Unité Physiopathologie et Génétique du Neurone et du Muscle, Univ Lyon, CNRS 5261, INSERM U1315, Université Claude Bernard Lyon 1, Lyon, France.
6. Research Institute for Sport and Exercise, University of Canberra, Canberra, ACT 2617, Australia.
7. University of Coimbra, Research Center for Sports and Physical Activity, Faculty of Sports Science and Physical Education, Coimbra, Portugal.
8. Department of Exercise Physiology and Sports Therapy, Institute of Sports Science, Justus Liebig University Giessen, 35394 Giessen, Germany.
9. Facultad de Deportes Campus Ensenada, Universidad Autónoma de Baja California, México

ABSTRACT

Background: The term immunometabolism describes cellular and molecular metabolic processes that control the immune system and the associated immune responses. Acute exercise and regular physical activity have a substantial influence on the metabolism and the immune system, so that both processes are closely associated and influence each other bidirectionally.

Scope of review: We limit the review here to focus on metabolic phenotypes and metabolic plasticity of T cells and macrophages to describe the complex role of acute exercise stress and regular physical activity on these cell types. The metabolic and immunological consequences of the social problem of inactivity and how, conversely, an active lifestyle can break this vicious circle, are then described. Finally, these aspects are evaluated against the background of an aging society.

Major conclusions: T cells and macrophages show high sensitivity to changes in their metabolic environment, which indirectly or directly affects their central functions. Physical activity and sedentary behaviour have an important influence on metabolic status, thereby modifying immune cell phenotypes and influencing immunological plasticity. A detailed understanding of the interactions between acute and chronic physical activity, sedentary behaviour, and the metabolic status of immune cells, can help to target the dysregulated immune system of people who live in a much too inactive society.

Corresponding author:

Department of Cell Biology and Development, Institute of Biomedical Sciences, University of São Paulo (USP), Av. Lineu Prestes, 524-lab.435, São Paulo, SP 05508-000, Brazil.
Email: josecesar23@hotmail.com

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Abbreviations: AMPK: 5' AMP-activated kinase; mTORC1: mTOR complex 1; HIF-1α: hypoxia inducible factor-1α; PPAR-γ: peroxisome proliferator-activated receptor gamma; ATP: adenosine triphosphate; PI3K: phosphoinositide 3-kinase; IFN-γ: interferon gamma; mTOR: mammalian target of rapamycin; IL: interleukin; CD: cluster differentiation; LPS: lipopolysaccharide; TLR-4: toll like receptor 4; TCR: T cell receptor; BCAA: branched-chain amino acids; PGC-1α: peroxisome proliferator-activated receptor-gamma coactivator 1 alpha; NF-kB: nuclear factor kappa B; AP-1: activator protein 1; STAT: signal transducer and activator of transcription; PBMC: peripheral blood mononuclear cells; WBC: white blood cells; TGF-β: transforming growth factor beta; FFA: free fatty acids; UPR: unfolded protein response; OXPHOS: oxidative phosphorylation; TNF-α: tumor necrosis factor alpha; CRP: C-reactive protein; BMI: body mass index; TAK: transforming growth factor-B-activated-kinase; TAB: TAK1-binding protein-1; IKK: IkB kinase; JNK: c-Jun N-terminal kinase; IRS: insulin receptor substrates; NLRP3: pyrin domain-containing protein 3; LDL: low density lipoprotein; ER: endoplasmic reticulum stress; HSP: heat shock proteins; MAPK: mitogen-activated protein kinases.

INTRODUCTION

The term "immunometabolism" was coined in 2011, describing an emerging research topic focused on understanding the metabolic pathways used by immune cells in response to challenges (e.g., pathogen exposure, inflammation), and their ability to crosstalk with metabolic tissues [1]. Immune cells show high metabolic flexibility with different subsets of lymphocytes, monocytes/macrophages, and neutrophils exhibiting deep metabolic alterations when moving from the resting to the activated state. Both the aging process and numerous infectious diseases, as well as diseases accompanied by chronic inflammation, are associated with changes in the metabolic function of leukocytes [2,3]. For example, HIV and COVID-19, cancer, cardiovascular and neurodegenerative diseases, obesity, and type 2 diabetes are morbidities associated with dysregulation in the metabolic response of immune cells. This, in turn, causes metabolic derangements in immune cells leading to a 'vicious cycle' of inflammation and disease progression [4–6]. Various lifestyle-associated diseases with an excess or lack of nutrients are associated with a modified immune response. Chronic hyperglycemia associated with excess release of amino acids and fatty acids into the circulation causes a chronic activated immune system [7,8]. In many conditions including SARS-COV-2 infection, an excess of nutrients (especially fats and glucose) reduces the ability to immune cells to resolve inflammatory conditions potentially increasing the likelihood of immunological overreaction, which might favor massive and unregulated cytokine secretion (a "cytokine storm") [9]. On the other hand, undernourishment can also be associated with a poor immune response given the importance of nutrients for basic functions of immune cells including proliferation [10]. Consequently, an adequate supply of substrates and nutrients is important for the balanced control of immunometabolism [11,12].

Prolonged physical inactivity is associated with a reduction in life expectancy and quality of life [13,14]. Physical inactivity favors development of visceral adiposity, and causes imbalances in the production of adipokines, myokines, hepatokines and other mediators of inflammation [15]. Sedentarism is considered a catalyst for development of low-grade inflammation associated with various lifestyle diseases. In contrast, a physically active lifestyle can positively stimulate various aspects of immune function on a clinical, cellular, and molecular level to reduce low-grade inflammation [16]. Acute exercise (especially aerobic exercise) induces the release of multiple signaling molecules (e.g., cytokines, hormones) and alters substrate and nutrient concentration in the immune cell environment [17]. Particular importance is attached to the secretome of skeletal muscles. For this purpose, the term "exerkines" was introduced as a collective term, covering any biomolecules such as peptides, metabolites and RNAs, secreted into circulation by tissues in response to exercise [18].

Regular exercise contributes to a balanced immunological state that can serve as an effective countermeasure against the development of chronic low-grade inflammation, particularly in older adults and those living with obesity and/or metabolic disease [19]. Understanding how exercise affects immunometabolism is a recent scientific endeavor, and there is a critical need to ascertain the metabolic processes and pathways by which the immune system responds to acute exercise and adapts to exercise training. The immunometabolic rearrangement of lymphocytes and macrophages in well-trained humans may have the potential to regulate immune response in chronic inflammatory diseases, such as obesity, cancer, cardiovascular and neurodegenerative diseases, and to delay immunosenescence.

In this review, we examine the complex interplay between immunometabolism and exercise with a focus on T cells and macrophages, and present new challenges and opportunities for this field. Our focus here ranges from the acute effects of single bouts of exercise, to the chronic effects of regular physical activity, and exercise training for fitness and sports. We review the immunometabolic regulators in immune cells (AMPK, mTORC1, HIF-1α and PPAR-γ) and examine the role and potential impact that exercise can have on the metabolism and function of lymphocytes and macrophages, and their interplay with skeletal muscle. Finally, we discuss the impact of sedentarism on immunometabolism, and how these effects can potentially be counteracted with exercise to reduce cardiometabolic risk factors and immunological aging. We examine the effect of both acute exercise (in the hours after a single bout of exercise), and the long-term effects of exercise training and regular physical activity on immunometabolism. Concepts in immunometabolism are inherently interdisciplinary and can be viewed from the perspective of how alterations in: a) molecular metabolic pathways and sensors within immune cells; and/or b) the global metabolic environment and impact immune cell function. In this review we have attempted to balance and integrate these perspectives, highlighting key molecular signaling pathways and how they may relate to, or be influenced by, whole-body metabolic changes initiated by changes in exercise, sedentary time, chronic disease, and aging.

IMMUNOMETABOLIC SENSORS

Molecular regulation of immunometabolism is associated with sensors that control the metabolic routes, inflammatory mediators, and differentiation and function of immune cells. Thus, the immunometabolic sensors are an important bridge that link metabolism and immunology particularly in lymphocytes and macrophages. Nutrient sensors are largely modulated by different intra- and extra-cellular concentrations of glucose, amino acids and fatty acids (and their intermediary metabolites). The levels of nutrients that immune cells are exposed to influence different intracellular energetic sensors, principally AMP-activated protein kinase (AMPK), mammalian target of rapamycin (mTOR), hypoxia inducible factor-1α (HIF-1α) and peroxisome proliferator-activated receptors (PPAR) [8].

AMPK

AMPK is an enzyme that acts as the energetic sensor of eukaryotic cells. A low ATP:AMP ratio increases AMPK activity [20], and subsequently fatty acid oxidation and ATP generation by the electron transport chain [21]. Moreover, AMPK inhibits the PI3K/AKT/mTOR pathway, essential for aerobic glycolysis. Immune cells in resting form (naïve T Lymphocytes and memory T or B lymphocytes) show strong activation of AMPK [22].

It is well established that AMPK is activated in different tissues, such as skeletal muscle, liver and adipose tissue after acute exercise and regular exercise training. While there are limited data for AMPK regulation after acute exercise or in regular exercise training in leukocytes, various regulatory effects of AMPK in immune cells have been shown. During T cell receptor (TCR) and CD28 co-stimulation in T lymphocytes, the activity of AMPK is upregulated. This increase in AMPK activation after TCR stimulation is fast, and then blunted during the effector phase [23]. Activation of AMPK reduces the release of IFN-γ by T effector lymphocytes. Moreover, AMPK is important for the fate of T cells. The reduction of AMPK and increase in mTOR induces the differentiation of the effector subset of CD8 lymphocytes, while an increase in AMPK and reduction in mTOR lead to long-lived memory CD8 T cells. Regarding CD4 cells, activation of AMPK induces the production of regulatory T cells (Tregs) and inhibits the differentiation of Th17 lymphocytes. Moreover, IL-10 induces AMPK activation particularly after acute bouts of exercise [24,25]. In this framework, well-trained subjects can have a lower Th1:Th2 ratio, which represents an aspect of the anti-inflammatory T cell profile specifically promoted by regular endurance training [26].

In terms of B cells, AMPK signaling is essential to mitochondrial homeostasis. For this cell type, a loss of AMPK can reduce antibody production. Accordingly, AMPK signaling appears to be of particular importance, especially for long-lived memory B cells [27]. A physically active lifestyle can enhance antibody production after vaccination in older adults [28]. The discovery of mechanisms associated with this improvement is important for new adjuvant therapies to increase the immunization response in the elderly [28,29].

In macrophages, activation of AMPK is an important mechanism to induce a shift to acquire the alternative and anti-inflammatory phenotypes (formely named M2), that are characterized by the production of anti-inflammatory mediators (IL-10, and IL-1ra) and growth factors (TGF-beta) [30]. Several anti-inflammatory drugs, such as salicylates, metformin, and corticosterone, blunt an inflammatory response and induce the acquisition of an anti-inflammatory/restorative phenotype by activation of the AMPK pathway [31–33]. Particularly, activation of dopaminergic signaling (more specifically by the D1 receptor) induces AMPK activation, leading to a shift in macrophage inflammatory phenotype [34].

mTORc1

mTOR is a highly conserved protein complex that can phosphorylate different targets [35], which exists in two sub-complexes called mTORC1 and mTORC2. mTORC1 is an energetic sensor that acts in opposition to AMPK [36], and is positively regulated by amino acids (e.g., leucine) and growth factors via the PI3K/AKT pathway [35]. Moreover, mTORC1 is a complex protein that induces the anabolic pathways and participates in the regulation of lipids, protein, and glycogen synthesis.

Aerobic glycolysis is critical for sustaining the effector functions of lymphocytes, which is in turn dependent on mTORC1/HIF-1α (Nieman and Pence 2020). mTORC1 deletion in CD4 lymphocytes reduces their proliferation and impairs their effector functions [22]. Moreover, mTORC1 inhibition increases Foxp3 activity and Treg numbers [37]. mTORC1 is essential for the differentiation of naïve T lymphocytes into CD4 and CD8 T effector lymphocytes, as well as antibody production by B lymphocytes [38].

The knowledge of the role of mTORC1 is inconclusive regarding the polarization to classic or alternative phenotype. Glycolysis stimulated by the mTORC-1/HIF-1α pathway is essential for mounting a pro-inflammatory response and production of reactive oxygen species [39]. Moreover, LPS-TLR4 canonical signaling, nutrient excess by inflammasome, and pro-inflammatory cytokines, all induce mTORC-1 [39,40]. While mTORC-1 is considered an essential player for classically activated macrophages (M1), that produce pro-inflammatory cytokines (IL-1β, TNF-α) and lipid mediators (PGE2), specific deletion of raptor can block mTORC1 pathway. Macrophages treated with rapamycin exhibit a more inflammatory phenotype, and show impaired acquisition of the alternative phenotype [41,42]. High intensity exercise decreases the circulating levels of branched chain amino acids (BCAAs) in endurance athletes [43]. It is well established that BCAAs can activate the mTORC. Therefore, reduced BCAA availability could also facilitate reduced immune cell activity [40]. Glutamine activates mTORC1 through a Rag GTPase-independent mechanism [44]. Serum glutamine is reduced after exhaustive exercise, and supplementation of endurance athletes with BCAA mitigates the reduction in glutamine. Furthermore, BCAA administration can divert cytokine production towards Th1 type response after exercise [45].

In summary, the maintenance and fine-tuning of mTORC-1 signaling is necessary, whereas chronic activation or deletion/inhibition of mTORC1 induces a pro-inflammatory response, and in combination with other players, this sensor is an important determinant of immune cell fate. mTORC-1 and HIF-1α are essential for activation of aerobic glycolysis and thus induces and supports inflammation. mTORC-1 and PPAR-γ/PGC1α are important for reprogramming the metabolism mediated by mitochondrial biogenesis and anti-inflammatory responses [46]. The type, intensity and duration of exercise are likely important in regulating mTORC activation in immune cells by modifying substrate availability.

HIF-1α

HIF-1 is a family composed by three isoforms of α and β subunits of a heterodimeric transcription factor that senses cellular oxygen [47]. HIF-1 is expressed in most immune cell types and essential for immunometabolic flexibility and the inflammatory response [48]. mTORC-1/HIF-1α pathway is essential to trigger glycolysis during TCR activation in CD4 lymphocytes, and cytotoxic activity of CD8 lymphocytes [49,50]. Furthermore, HIF-1α is necessary to induce the Th17 differentiation, while inhibition of this transcription factor is observed in Tregs [47].

HIF-1α is essential in development of B cells. Given that the germinal centers of B cells are hypoxic, HIF-1α plays a role in the induction of B cell development [51]. Moreover, HIF-1α is necessary for IL-10 production and CD-11b expression in B cells, and lack of HIF-1α reduces the anti-inflammatory signaling between B cells and dendritic cells, otherwise promoting inflammation [47,52].

HIF-1α deletion reduces ATP levels and bactericidal function in macrophages stimulated by LPS [53]. Macrophages can function in a hypoxic environment, but this ability is depen-

dent on HIF-1α expression. In normoxic conditions, LPS stimulation promotes HIF-1α nuclear activity with upregulation of the glycolytic and pentose phosphate pathways (PPP). Moreover, overexpression of HIF-1α induces the M1 phenotype [54]. While succinate, an intermediary of the Krebs cycle, induces HIF-1α stabilization and M1 activation of macrophages [55], itaconate, generated by catabolism of cis-aconitate in mitochondria, inhibits the HIF-1α pathway and activates the alternative phenotype of macrophages [56,57].

Most data regarding the regulation of HIF-1α during exercise has come from studies of skeletal muscle. Within this tissue, acute bouts of exercise stabilize HIF-1α, and HIF-1α response is blunted during long-term exercise training, as part of a local physiological response to exercise [58]. The effect of acute or chronic exercise on HIF-1α expression in leukocytes remains uncertain. Elevated serum IL-10 after high volume training [25] increases CD11b expression by monocytes [59], contributing to the notion that physical fitness could modulate the effects of HIF-1α on monocytes. However, another study did not find differences in monocytes between low and high VO2max subjects, pre or post-acute exercise session, with and without LPS stimulation [60].

PPAR-γ

PPARs are a family (PPAR-α, PPAR-β and PPAR-γ) of transcription factors responsible for regulating lipid metabolism (lipogeneses, lipid mediators, synthesis, and fatty acid oxidation) [61]. The three different isoforms of PPARs exert various regulatory roles in immune cells. However, the most studied isoform is PPAR-γ and its effects on metabolism of immune cells. Expression of PPAR-γ is increased in both lymphocytes (Th2) and macrophages (M2) [62,63]. Although the fatty acid synthase and de novo lipogenesis is essential in early effector response of lymphocytes after TCR stimulation, this step is regulated by mTORC1/SREBP axis and not affected by PPAR-γ deletion in T cells [64]. On the other hand, the activation of PPAR-γ impairs the clonal expansion of effector T cells [65].

The maintenance of fatty acid metabolism necessary to promote the effector function of Th2 cells is dependent of PPAR-γ [62]. AP-1, STAT5, GATA-3, IL-5 and IL-13 are all target genes of PPAR-γ in Th2 cells [66]. PPAR-γ is necessary to increase the fatty acid uptake, lipolysis and glycolysis in Th2 cells. Activation of PPAR-γ activates downstream mTORC1 in this lymphocyte subset [64]. For Tregs, PPAR-γ is not essential for differentiation of all its subsets. However, the expression of FOXP-3 and Treg differentiation is dependent of PPAR-γ expression in Tregs residents on adipose tissue and deletion of this subset by specific KO of PPAR-γ on Foxp3 flox, and induces a range of metabolic disturbances [67]. Moreover, agonist or inhibitor effects of PPAR-γ reduce the expression of Foxp3, indicating that PPAR-γ expression should be within the specific range for Foxp3 activation and lymphocyte differentiation into Tregs [68]. PPAR-γ induces the expression of genes that increase mitochondria biogenesis and oxidative metabolism, and reduces the transcription of pro-inflammatory genes by the repression of NF-kB, AP-1 and STAT family [69].

Concerning exercise, moderate aerobic trained mice with PPAR-γ deletion in myeloid cells show a reduced subset of macrophages expressing CD206 in subcutaneous adipose tissue macrophages as compared with trained wild type mice [70]. In obese mice, despite the absence the PPAR-γ expression in myeloid cells, aerobic training increases the concentration of anti-inflammatory cytokines, but the expression of surface markers (e.g. CD206) remained low as observed in non-obese mice [61]. Finally, PPAR-γ expression in monocytes appears to be dependent of physical fitness level, given that monocytes from high VO_2 max subjects show increased PPAR-γ mRNA after LPS stimulation [60]. An anti-inflammatory profile of macrophages is observed in endurance athletes and may be induced by PPAR-γ, although further confirmation is required. In the same study, rosiglitazone (PPAR-γ agonist) increases mRNA expression of AMPK in monocytes, providing evidence that these two energetic sensors may act synergistically [60].

THE COMPLEX ROLE OF EXERCISE ON THE METABOLIC FUNCTION OF IMMUNE CELLS

A recent multi-omics approach indicates intensive crosstalk between immunity and metabolism during exercise [71]. Various molecules are affected by acute exercise and T cells are highly sensitive to changes in their microenvironment [72]. There is emerging evidence that exercise-induced alterations directly and indirectly affect T cell immunometabolism.

Effects of exercise on T cell metabolism

Exercise training improves TCR activation signal and increases the expression of Zap70 in peripheral blood mononuclear cells (PBMC)s [73]. Correspondingly, intracellular calcium stores and proliferative capacity of CD3+ cells are increased by chronic voluntary exercise in mice [74]. A potential mechanism is that exercise itself induces a mild T cell activation, as increased expression of CD25, a typical activation marker of T cells, has been reported in humans after acute moderate intensity endurance and resistance exercise combined, and resistance exercise alone [75]. Another explanation is that an acute bout of exercise activates the TCR and the TCR signal quality associated with increased body temperature [76]. Overall, an effect of exercise on TCR activation and function is likely but remains to be shown conclusively.

Depending on the exercise type, intensity, and duration, cytokine signatures and substrate availability can be affected [25,77]. TGF-β1 [78], IL-6 [79], IL-1β, and IL-23, which are released in response to muscle damage, play an essential role in T cell metabolism and differentiation [80]. Lactate accumulation, which increases after acute bouts of high-intensity exercise, increases STAT3 activity followed by increased Th17 polarization of lymphocytes [81]. Similarly, endurance exercise alters the Th17/Treg balance towards Th17 [82].

During exercising in a fasted state or for a long duration without adequate carbohydrate supplementation, a very large neuroendocrine response is observed to ensure that the blood glucose level is maintained. Thus, we observed the impact on immune-neuroendocrine response by elevation in cortisol levels that induces an immunosuppressive response [83]. Moreover, the levels of glucose, as one of the main substrates of T cells, facilitates T-cell activation. While blood glucose levels can remain relatively constant, however, it is possible that the

glucose and amino acids levels are reduced in lymphoid organs, whereas the blood flow is reduced to central organs to provide the increase of blood flow in skeletal muscle, although this hypothesis has yet been evaluated fully. Therefore, during low glucose conditions, activated naïve T cells and central memory T cells reduce their function, including lowering of IFN-γ expression. During conditions of glucose deprivation, these cells increase OXPHOS and fatty acid metabolism by upregulating the expression of the FFA transporter CD36, increasing autophagy, and utilizing glutamine for biosynthesis [84]. However, athletes may be at higher risk for immunosuppression at the onset of overtraining, when plasma glutamine concentrations decrease, such as after 8 weeks of high-intensity interval exercise [77]. Glutamine, next to glucose, is essential for the hexosamine biosynthesis of T cells and for their proliferation [85]. Exercising in a well-nourished state should not put athletes at a higher risk of immune dysregulation by maintaining the availability of substrates for T cell activation.

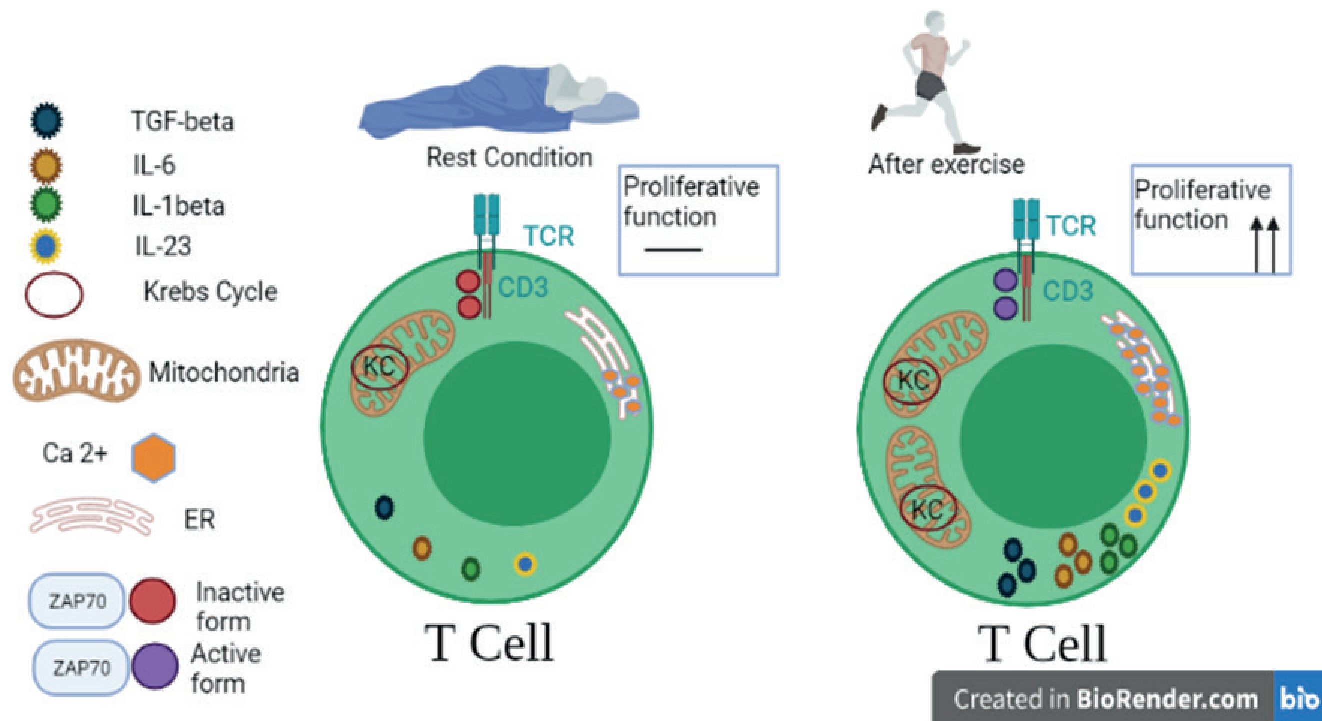

Figure 1. Molecular mechanisms that influence T cell metabolism during acute stress.

Besides macrophages and neutrophils, the anti-tumoral effect of acute and chronic exercise is largely dependent on the CD8 cytotoxic T cells [86]. Moreover, acute exercise modifies metabolites in the circulation in both humans and mice. Addition of malate, succinate and fumarate reduces the expression of the surface marker CD62L on cytotoxic CD8. Low expression of CD62L occurs after TCR activation of the CD8 cytotoxic T cells [86]. Moreover, lactate induces the migratory capability and increases granzyme B production in a dose-dependent manner in CD8 cytotoxic cells. Finally, in a murine model of cytotoxicity induced in an ovalbumin vaccination model, 20 min of aerobic exercise increases pyruvate and alpha ketoglutaric acid derived from glucose metabolism and consequently improvement the cytotoxic CD8 cells response after vaccination [86]. Remodeling of metabolism in lymphocytes may be important in the anti-tumoral response induced by acute exercise. A proposed model of mechanisms supporting future research in exercise immunology is presented in Figure 1.

Effects of exercise on macrophages

The role of metabolic plasticity in the fate of macrophage phenotype is well characterized. While classical macrophage activation is induced by aerobic glycolysis, the alternative phenotype is induced by a proportional increase in oxidative metabolism [87]. Moreover, different macromolecules and metabolites, which are affected by acute exercise, are potent regulators of macrophage function [8,88,89]. Lactate-treated macrophages show a reduction in glycolysis and an increased fatty acid oxidation. Moreover, they show increased phagocytic function against Mycobacterium Tuberculosis and reduced TNF-α and IL-1ß production after LPS stimulation [90]. Besides lactate, glutaminolysis is essential for efficient clearance of apoptotic cells [91]. Studies also show that amino acids and several fatty acids are immunomodulating agents for macrophages [92,93]. Mode, intensity, duration of exercise, and fitness levels can differentially modify cytokine secretion and availability of various metabolites. Understanding the detailed metabolic effects these changes have on macrophages will further characterize mechanistic links between exercise and immune function [71].

The anti-inflammatory effects of aerobic exercise training and its role in the treatment and prevention of metabolic and chronic inflammatory diseases is influenced by macrophage function. The first step for macrophage activation is chemotaxis of monocytes. Data show that improvement in physical fitness mitigates the migration of pro-inflammatory monocytes in patients with central obesity [94]. Aerobic exercise training promotes alternative phenotypes and decreases classical macrophages in the obese adipose tissue [95]. A 16-week program of treadmill exercise, in high fat diet (HFD) treated mice, de-

creases CD11c gene expression (pro-inflammatory marker) and increases CD163 (anti-inflammatory marker) in adipose tissue macrophages [95]. Both continuous and interval aerobic training induce pro- to anti-inflammatory transition and increase the total number of anti-inflammatory macrophages in adipose tissue of high fat diet (HFD)-treated rats. However, in the same study, interval training was more effective than continuous training [96].

tion of mitochondrial biogenesis and increased mitochondrial activity in immune cells via β-AR stimulation [97]. Mice that receive daily catecholamine injections, increase the anti-inflammatory phenotype in peritoneal macrophages, and enhance IL-10 production [98]. Interestingly, obese animals show a reduction in β-ARs in comparison with lean exercised mice [99] (Figure 2).

In summary, acute and chronic aerobic exercise may ex-

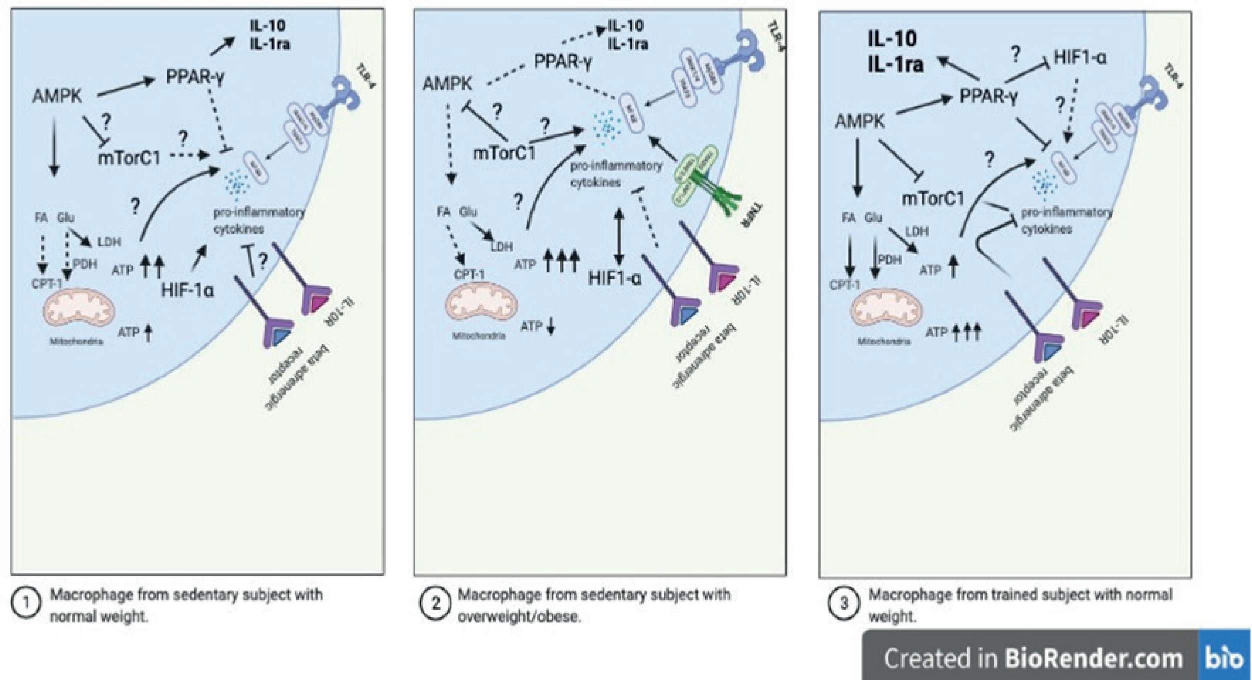

Figure 2. Metabolic profile of macrophages and its adaptations in dependence on physical fitness and body composition. FA = fatty acid; Glu = glucose; PDH = pyruvate dehydrogenase; LDH = lactate dehydrogenase; ATP = adenosine triphosphate; CPT-1 = carnitine palmitoyltransferase 1; mTORC1 = mammalian target of rapamycin complex 1; AMPK = AMP-activated protein kinase; PPAR-γ = peroxisome proliferator-activated receptor γ; TLR-4 = Toll like receptor 4; TNFR = Tumor Necrosis factor receptor; IL-10 = interleukin 10; IL-1ra = interleukin 1 receptor antagonist; HIF-1 alpha = hypoxia-inducible factor 1-alpha; MYD88 = Myeloid differentiation primary response protein; IRAK1/4 = interleukin-1 receptor-associated kinase 4; TRAF6 = TNF Receptor Associated Factor 6; NF-kB = factor nuclear kappa B; TRADD = Tumor necrosis factor receptor type 1-associated DEATH domain protein; cIAP1/2 = encoding a copper-transporting ATPase; TRAF2/5 = TNF receptor-associated factor 2.

The mechanisms behind exercise-induced changes in macrophage polarization and metabolism remain elusive. The ability of aerobic exercise training to promote an anti-inflammatory phenotype in obese adipose tissue macrophages is lost when mice have selective deletion of PPAR-γ in the myeloid lineage [61]. Thus, it seems PPAR-γ plays an important role in switching macrophage metabolic and phenotype induced by aerobic exercise training. The same group showed that lean sedentary mice, lacking PPAR-γ in the myeloid cells, have an exacerbated pro-inflammatory profile. An 8-week intervention of treadmill, moderate aerobic training (continuous exercise 70% Vo2 max) promotes the anti-inflammatory phenotype of adipose tissue macrophages but not peritoneal macrophages [70]. It appears that aerobic exercise training plays distinct roles in macrophage phenotype and metabolism, depending on the basal level of inflammation.

One possible mechanism by which aerobic exercise training alters macrophage polarization is catecholamine release. Both monocytes and macrophages express adrenergic receptors (ARs) and respond to catecholamine stimulation. During the exercise session there is release of adrenaline and promo-

ert immunomodulatory effects, improving pro-inflammatory macrophage response in healthy individuals, and inducing an anti-inflammatory phenotype in individuals with higher basal levels of inflammation. However, the molecular mechanisms behind this immunomodulatory effect remain unclear.

IMMUNOMETABOLIC DYSREGULATION IN SEDENTARY BEHAVIOR AND INCREASED RISK OF CARDIOMETABOLIC DISEASE: THE PROTECTIVE ROLE OF EXERCISE.

We can define sedentary behavior as an energy expenditure of ≤1.5 metabolic equivalents of task (METs). Physical activity as any body movement generated by the contraction of skeletal muscles that raises energy expenditure above resting metabolic rate, and physical inactivity represents the non-achievement of physical activity guidelines [100].

Engaging in physical activity elicits many health benefits, including a reduced risk of cardiometabolic disease (e.g.,

type 2 diabetes, atherosclerosis, hypertension). A key underlying mechanism appears to be exercise-mediated reduction in chronic low-grade inflammation, which is a key contributor to development of insulin resistance and vascular endothelial dysfunction [19]. Conversely, sedentary behavior is associated with various adverse metabolic and health-related outcomes, independently of physical activity levels [101,102]. Given the widespread prevalence of sedentary lifestyles and inadequate physical activity levels [103], it is important to understand how sedentary behavior impacts the interaction between inflammatory and metabolic pathways, as well as the potential of exercise to rescue immunometabolic defects arising from increased sedentary behavior. We propose that a perpetual cycle of immunometabolic dysregulation links sedentary behavior to increased cardiometabolic diseases, on the other hand, we suggest evaluating the potential of exercise to enable preventive or therapeutic effects.

Evidence supporting a link between sedentary behavior and immunometabolic dysregulation

Numerous epidemiological studies support a detrimental association between sedentary behavior, impaired metabolic outcomes (e.g. fasting/postprandial glucose, insulin sensitivity and FFAs) [104,105], and elevated serum/plasma concentrations of inflammatory markers (e.g. IL-6, IL-1β, CRP, TNF-α) [106–108]. Moreover, white blood cell (WBC) count – a potential biomarker of cardiometabolic disease risk [109] – increases linearly with sedentary time [110]. Allocating as little as 30 min of daily sedentary time to moderate-to-vigorous physical activity lowers circulating IL-6 and WBC [111]. The association between sedentary time and markers of chronic inflammation is attenuated when adjusting for body mass index (BMI) and/or waist circumference in some [105, 107, 112] but not all [104] studies. This outcome highlights the potential of sedentary behavior to impact inflammatory markers independent of adiposity. Despite these conflicting findings, it is generally accepted that adipose tissue dysfunction initiates immunometabolic derangements that lead to chronic inflammation [113,114]. A large proportion of the detrimental effects of sedentarism may be attributable to increases in visceral fat mass.

Intervention studies examining the impact of reducing sedentary time on markers of inflammation are currently limited. Reducing sedentary behavior by increasing walking time reduces levels of circulating IL-6 and monocytes, and also lowers LPS-stimulated cytokine production [115]. Overweight/obese individuals who replaced prolonged sitting with light intensity activity show a gene expression shift towards an anti-inflammatory profile in both adipose tissue [116] and skeletal muscle [117]. Despite the paucity of research examining the impact of reducing sedentary behaviors on inflammatory markers, studies consistently demonstrate clinically meaningful improvements in various metabolic parameters including glycemia, insulin sensitivity and blood lipids [118–120]. Combining evidence from cross-sectional analyses and experimental studies supports the hypothesis that the detrimental effects of sedentary behavior on inflammatory outcomes are driven by metabolic derangements.

Mechanisms by which sedentary behavior fuels the vicious cycle of metabolic dysfunction and inflammation

Long-term physical inactivity favors the development of visceral adipose tissue characterized by a shift towards a pro-inflammatory adipocyte profile related to accumulation of pro-inflammatory macrophages, neutrophils, B lymphocytes and CD8+ T lymphocytes [121]. Local release of pro-inflammatory cytokines, such as TNF-α, directly stimulates adipose tissue lipolysis while reducing the inhibitory effect of insulin on lipolytic enzymes The ensuing spillover of FFAs and pro-inflammatory cytokines into systemic circulation induces ectopic lipid accumulation and insulin resistance in vital organs/tissue (e.g., liver, skeletal muscle), exacerbating fasting and post-prandial hyperglycemia and hyperlipidemia [113,114]. Importantly, elevated blood glucose and FFA levels directly impair immune cell function causing the release of inflammatory mediators that further propagate systemic low-grade inflammation. On the other hand, the systemic depletion of CD4 cells improves glucose tolerance while leaving insulin sensitivity, adipose tissue morphology, adipose tissue macrophage activation, and adipose tissue macrophage proliferation as signs of adipose tissue dysfunction unaffected [122].

Toll-like receptors (TLRs) have emerged as key integrators of the relationship between metabolic dysfunction and chronic inflammation-induced insulin resistance. TLR2 and TLR4 are involved in both innate and adaptive immune responses. These receptors are typically activated by bacterial cell wall lipids, but also respond to host lipids and other damage-associated molecular patterns, playing an important role in non-infectious (sterile) inflammatory responses [123]. Activation of TLR2/4 promotes the association of transforming growth factor-B-activated-kinase 1 (TAK1) with TAK1-binding protein-1 (TAB1) activating IkB kinase (IKK) and the c-Jun N-terminal kinase (JNK). IKK and JNK activation induces pro-inflammatory cytokine expression via nuclear-factor kappa B (NF-kB) and impairs insulin action via serine phosphorylation of the insulin receptor substrates (IRS1/2) [114]. TLR4 activation is also a key event in priming the NLRP3 inflammasome, thereby contributing to NLRP3 inflammasome formation and the caspase-1-dependent release of the pro-inflammatory cytokines IL-1β and IL-18 [124].

In addition to innate immune responses, TLRs can also activate adaptive immune response to upregulate the expression of major histocompatibility complex II, a co-stimulatory molecule on antigen presenting cells, and promote the release of IL-12 from dendritic cells [125]. Although saturated FFAs have been traditionally implicated as the main TLR4 ligand through which metabolic overload drives inflammation and insulin resistance [126,127], recent evidence indicates that saturated FFAs do not directly activate TLR4. TLR4 can prime macrophages for FFA-dependent activation of pro-inflammatory pathways via alterations in cellular lipid metabolism and accumulation of lipid species [128]. Despite the controversial role of FFAs in TLR4 activation, circulating levels of other TLR4 ligands (e.g., LPS, oxidized LDL) and TLR4 expression are also elevated in individuals with overweight/obesity and type 2 diabetes [129,130]. Collectively these effects provide additional mechanisms by which metabolic defects arising from sedentary behavior may drive inflammation.

Another proposed mechanism of inflammation in seden-

tarism and adiposity is endoplasmic reticulum (ER) stress. This stress occurs when the cellular protein folding capacity and calcium homeostasis of the ER are perturbed, and the unfolded protein response (UPR) activated to re-establish ER homeostasis [131]. The UPR has three effects: to inhibit protein translation that decreases the workload of the ER, to induce the expression of genes encoding chaperone proteins that increase the protein folding capacity of the ER, and to activate ER-associated degradation machinery to clear misfolded proteins [131]. Integral to the sensing of the ER stress and induction of the UPR are inositol-requiring protein-1 (IRE1), activating transcription factor-6 (ATF6), and RNA-dependent protein kinase (PKR) – like ER kinase (PERK) [131]. Importantly for inflammation-induced insulin resistance, activation of the UPR leads to nuclear translocation of NF-kB thereby priming the NLRP3 inflammasome and inducing expression of pro-inflammatory cytokines that impair insulin signaling [132,133]. Moreover, IRE1 and PERK can induce serine phosphorylation of IRS1/2 via JNK and IKKB [132–134], providing an additional mechanism by which ER stress induces insulin resistance [132]. Taken together, metabolic overload due to higher FFAs and glucose increases ER stress in several tissues provides an additional mechanism by which sedentarism drives immunometabolic dysfunction. There is also evidence that interventions that decrease body fat can reduce ER stress and improve insulin signaling [133].

Interleukin-6 (IL-6) is the prototypical myokine released from contracting skeletal muscle in proportion to exercise volume and intensity, thus the long-endurance exercise with high intensity, as marathon and triathlon induce the huge increase of IL-6 into circulation. IL-6 binds to its monocyte membrane-bound receptor to upregulate anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist (IL-1Ra) [134]. IL-6 infusion in humans increases circulating levels of IL-10 and IL-1Ra [135]. IL-10 is also produced by contracting skeletal muscle in response to acute endurance exercise and released into the vasculature independent of IL-6 [136]. Contracting skeletal muscle also releases heat shock proteins (HSP) to maintain cellular homeostasis during acute exercise [137,138] and chronic increase of circulating HSP 60 and 70 levels are associated with a decrease in monocyte TLR4 expression, subsequently downregulating the secretion of pro-inflammatory cytokines TNF-α and IL-1β [139]. Given that sedentary behavior is associated with prolonged periods of inactivity, the absence of skeletal muscle contraction (and thus the lack of release of the anti-inflammatory molecules) could partly explain how sedentary behavior drives immunometabolic dysfunction.

The chronic energy surplus that accompanies sedentary lifestyles may also reduce anti-inflammatory cytokine action. The ability of IL-10 to inhibit TNF-α production is reduced in cells from individuals with type 2 diabetes (T2D), and in macrophages grown in high-glucose media [140]. This defect in IL-10 action likely involves diminished STAT3 phosphorylation, and is restored when cells are treated with a small molecule activator of the inositol phosphatase SHIP1 [140]. Thus, loss of anti-inflammatory cytokine action due to nutrient surplus represents another mechanism by which a sedentary lifestyle may exacerbate inflammation-induced insulin resistance. The concept of immune cell dysfunction under metabolic overload is consistent with experiments demonstrating that hyperglycemia and hyperlipidemia induce TLR-dependent inflammation in human monocytes, thereby propagating a state of systemic inflammation [141,142].

Mechanisms by which aerobic training exercise interrupts the vicious cycle of metabolic dysfunction and inflammation

Given that sedentary behavior is associated with increased fat mass, and adipose tissue hypertrophy is a primary contributor to the initiation of chronic inflammation and insulin resistance, a reduction in visceral fat mass is a major mechanism by which exercise counteracts immunometabolic dysfunction. However, rodent studies demonstrate that aerobic exercise training may also prevent macrophage infiltration into adipose tissue, and induces a phenotypic shift in adipose tissue macrophage profile to an anti-inflammatory phenotype [95,143]. Although the ability of aerobic exercise training to reduce adipose tissue inflammation independent of fat loss remains to be established in humans [144], a decrease in adipocyte inflammation is presumably reflected by systemic elevations in adiponectin. Reductions in TNF-α, CRP, IL-6, and/or IL-8 are also apparent after an exercise training intervention in overweight/obese individuals. Importantly, the reduction in adipocyte size/inflammation, and subsequent decrease in circulating FFAs and pro-inflammatory mediators, should prevent development of inflammation and insulin resistance in circulating immune cells and metabolic tissues (e.g., muscle, liver). The improvement in systemic inflammation is presumably aided an exercise-mediated reduction in hepatic lipid content [145], and improved capacity of skeletal muscle to oxidize fatty acids [146,147]. These changes would prevent accumulation of macrophages and/or desensitization of insulin signaling cascades in these tissues [124].

A reduction in TLR4 expression and/or activation of immune cells provides an additional mechanism by which aerobic exercise counteracts the cycle of inflammation and insulin resistance arising from sedentarism. In support of this mechanism, both acute exercise and chronic training [148] can reduce TLR4 expression on monocytes in healthy human participants. Importantly, the exercise-mediated reduction on monocyte TLR2/4 expression is maintained in individuals at high risk for cardiometabolic disease, and individuals diagnosed with T2D [149]. These data indicate the ability of aerobic exercise to reduce TLR expression persists even in the presence of metabolic dysfunction. The precise mechanisms for reduced TLR expression with exercise remain unclear, but likely involve downregulation of TLR expression, TLR shedding and/or TLR internalization from the cell surface [19]. Exercise training also reduces various TLR ligands including FFAs [147] and oxidized LDL [150]. Circulating levels of fetuin – a hepatokine that promotes the effects of circulating lipids on TLR-mediated inflammation [151], and inversely correlates with improved glucose tolerance following training, are also reduced following training in humans [152]. These decreases in TLR expression and ligation likely mitigate activation of various downstream targets mediating pro-inflammatory cytokine production and insulin resistance [133].

The exercise-induced induction of the UPR is emerging as an important mediator of the adaptive response to aerobic exercise training [153,154]. Unresolved ER stress leading to

chronic activation of the UPR is associated with inflammation-induced insulin resistance in multiple tissues [131]. The ability of aerobic exercise to moderate ER stress via UPR activation represents an additional mechanism by which aerobic exercise interrupts immunometabolic dysregulation. Since weight loss-induced improvements in insulin sensitivity following gastric bypass surgery are associated with reduced markers of ER stress and JNK phosphorylation in adipose tissue, exercise-induced reduction in fat mass may mitigate ER stress [133]. However, reductions in p-IRE1, p-eIF2α (a surrogate marker of PERK activation), and p-ATF6 are observed following 3 months of training without corresponding changes in BMI or waist circumference in individuals with obesity [155], implying that exercise-mediated resolution of ER stress may be independent of fat loss. Evidence supporting the contribution of reduced ER stress to exercise-induced improvements in inflammation and insulin resistance is limited (Figure 3).

activation of the JAK1-STAT3 pathway [156]. Importantly, IL-10 counteracts insulin resistance in rodents [157,158] and correlates positively with insulin sensitivity in humans [159]. IL-1ra is a competitive inhibitor of signaling through the IL-1 receptor and blocks the effects of the classical pro-inflammatory cytokine IL-1β [124], which is a key contributor to obesity-induced insulin resistance [160]. The ability of IL-6 to stimulate IL-10 and IL-1ra production from immune cells likely explains why LPS-induced TNFα production is attenuated in exercising healthy males compared to resting controls [161]. In addition to IL-6, other myokines such as IL-7 and IL-15 are also implicated in control of immune cell function [162], and can mediate the anti-inflammatory effects of chronic and acute exercises (Figure 4)

Given that anti-inflammatory actions of IL-10 are impaired in cells from individuals with T2D and monocytes exposed to high glucose [140], and that hyperglycemia/hyperlipidemia induce immune cell inflammation/overactiva-

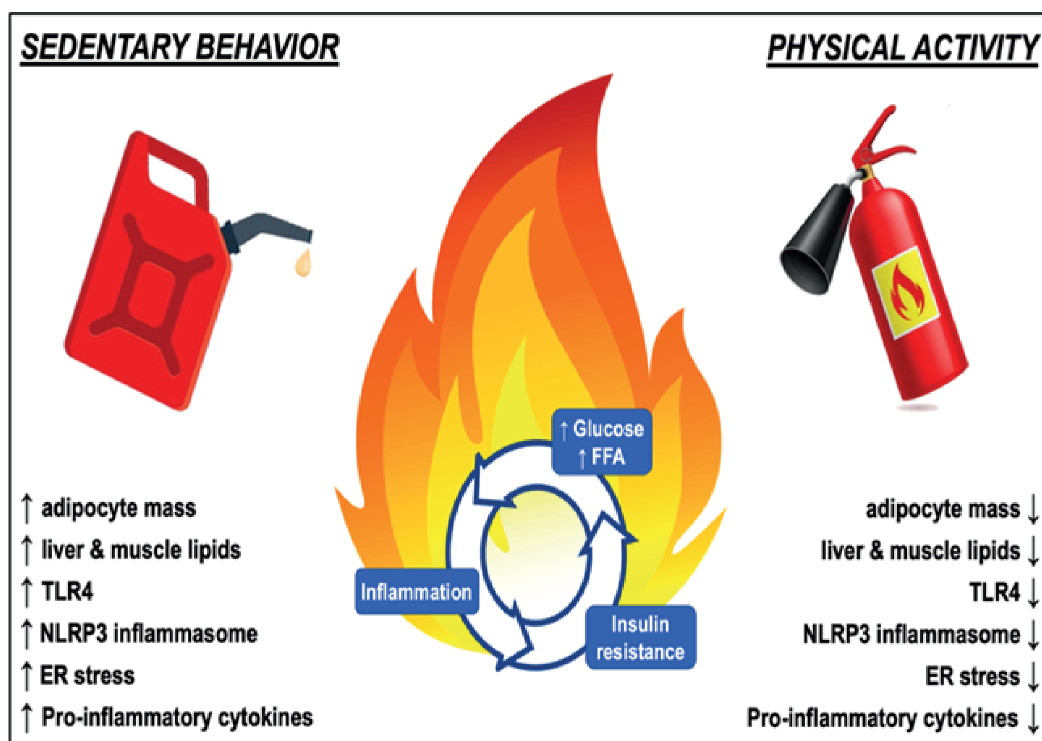

Figure 3. Vicious cycle of immunometabolic dysfunction that fuels cardiometabolic disease. Sedentary behavior feeds, and physical activity counteracts, the vicious cycle through multiple potential mechanisms.

Another mechanism of acute and chronic aerobic exercises that mitigates the vicious cycle in sedentary individuals is myokine production. The production and release of IL-6 from contracting skeletal muscle is widely implicated as a key mechanism underlying the anti-inflammatory effects of aerobic exercise [134]. Other mechanisms are likely involved as some patients cannot exercise at an intensity that leads to significant myokine release, yet experience significant anti-inflammatory benefits. Based on studies involving IL-6 infusion in healthy humans, the exercise-mediated increase in muscle-derived IL-6 purportedly mediates effects in IL10 and IL-1Ra [135] – molecules that exert potent anti-inflammatory effects. IL-10 is a prototypical anti-inflammatory cytokine released from various types of immune cells that inhibits production of pro-inflammatory mediators via

tion [141,142], exercise may counteract immunometabolism dysregulation via reduced post-meal blood glucose/lipid excursions. Several studies support the ability of short bouts of exercise centered around main meals (i.e., shortly before or after) to reduce hyperglycemia [163–165] in individuals at high risk for, or diagnosed with, T2D. Structuring exercise around meals may also be an efficacious strategy for breaking up prolonged bouts of inactivity. The impact of pre/post-meal exercise interventions aimed at lowering post-prandial glucose/lipid spikes on anti-inflammatory cytokine action remains unclear.

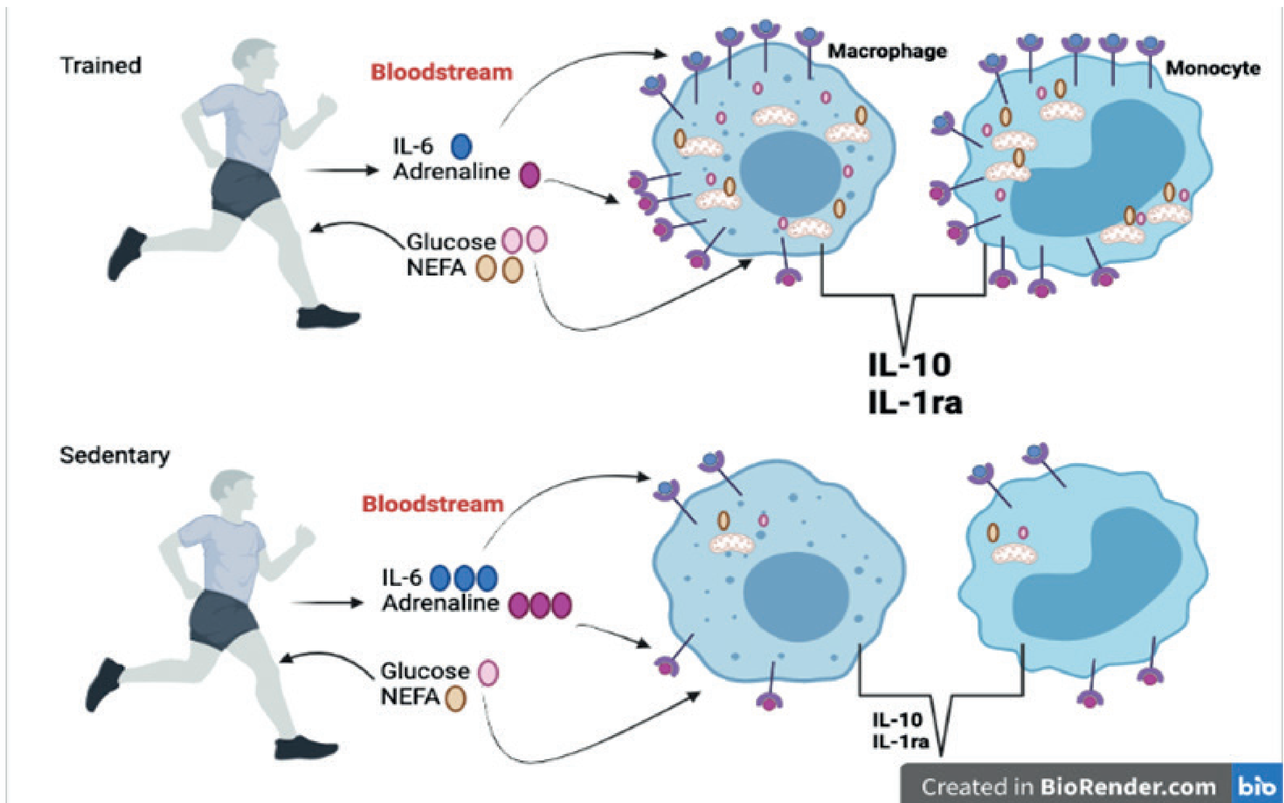

Figure 4. Effects of an acute bout of exercise session on monocytes/macrophages from sedentary and trained subjects. IL-6 = interleukin 6; NEFA = Non-Esterified Fat Acids; IL-10 = interleukin 10; IL-1ra = interleukin 1 receptor antagonist.

METABOLIC PERTURBATION OF IMMUNE CELLS DURING AGING, AND THE PROMINENT ROLE OF LIFELONG EXERCISE

Aging has profound impact on the immune system, affecting its capacity to mount robust immune responses, particularly T cell responses. Healthy aging depends on a complex gene-environment interaction culminating in a lower pro-inflammatory status, and a preserved humoral immune response [166]. Thymic involution is one of the main features of aging, and naïve T cells then have to rely on homoeostatic proliferation to maintain the peripheral T-cell pool. Characteristics of T-cell immunosenescence include an inverted CD4:CD8 ratio, low numbers and proportions of naive T-cells (that would impair the capacity of the immune system to deal with new pathogens), and larger numbers of effector memory T-cells in late stage of differentiation [167]. Other T cell changes with aging include diminished T cell receptor (TCR) clonal diversity, a more tolerogenic phenotype retained selectively [168], and declining ability of naïve and memory T cells to proliferate in response to TCR stimulation. T cell responses are impaired, which leads to poor vaccination efficacy and increased susceptibility to infections, cancer, and auto-immune diseases. Persistent infections, such as cytomegalovirus, further reduce the naïve T-lymphocyte repertoire and drive effector T-cells into senescence [169]. These senescent T-cells are apoptosis resistant, incapable of dividing, and thought to be the major cause of the chronic low-grade inflammation seen with age [170]. Older persons with a pronounced chronic low-grade inflammatory profile are more prone to frailty and mortality [171]. Age-related loss of Treg function would contribute to a greater risk of autoimmune disease, while an increase in Treg numbers could compromise immune responses, increasing the risk of malignancies and infection.

In aged T cells, signaling pathways linked to metabolism are dysregulated, for example, increased basal activation of the PI3K/Akt/mTOR and MAPK signaling pathways [172]. Inflammaging can lead to chronic PI3K/Akt/mTOR pathway activation, increased basal Glut1 expression, and glycolytic activity [173]. Aged senescent memory T cells can hyperactivate the MAPK pathway [174]. Increased activation of PI3K/Akt/mTOR and MAPK signaling pathways seem to be characteristic of T cell aging which increases the rate of glycolysis and mitochondrial mass [175].

Aging will also affect the metabolic machinery of the cells resulting in accumulation of dysfunctional mitochondria with damage to mtDNA [175], increased ROS production [176], decreased levels of NAD+ in the cell, and lower efficiency in metabolic pathways [177]. The role of mitochondrial dysfunction in aging-related progressive immune system alterations has been recognized [178], and exercise is considered an effective non-pharmacological strategy to counteract mitochondrial aging and dysfunction [179]. The crosstalk between exercise and the immune system is well known [180]. For example, hexokinase I activates the NLRP3 inflammasome leading to caspase 1 activation and processing of pro-IL-1b, while glyceraldehyde-3-phosphate dehydrogenase binds to mRNA encoding IFN-γ repressing its translation [181]. Cytokine-induced differentiation into Th1, Th2 and Th17 appears to depend on activation of the mTOR signaling pathway [37].

Regular bouts of exercise can offset T-cell immunosenescence by inducing apoptosis of senescent and functionally exhausted late stage differentiated T-cells [182,183]. A few studies have investigated the impact of lifelong exercise on inflammation

and immunosenescence, using a "master athletes' model". Master athletes, who maintain a healthy lifestyle, even in advanced age, represent an interesting cohort given many express a unique physiological phenotype that could be termed 'exceptionally successful aging' [184]. Master athletes show great motor skills and excellent body composition, and are predisposed to have a more efficacious immune defense, including stronger and longstanding antibody responses to the influenza vaccine, better immune-metabolic regulation, and redox balance, and attenuated biological age [185–187].

Master athletes usually have higher aerobic capacity values than age-matched non-athlete controls, and exhibit higher numbers of CD4+ naive T cells [185]. Individuals with a better physical fitness condition have fewer CD4+ and CD8+ T cells with a senescent/differentiated phenotype and lifelong exercise has been purported to limit accumulation of senescent T cells with age [183]. Lifelong training can also maintain the balance between pro- (e.g., TNF-α) and anti-inflammatory (e.g., IL-10) cytokines, and plasma IL-10 levels are similar to those of younger individuals [29]. Tregs numbers and activation (e.g., increased percentage of subjects expressing forkhead box P3 (FoxP3) and transforming growth factor beta (TGF-β) were also maintained as adaptive responses to lifelong training [188]. Lifelong athletes can show modifications in clock genes in CD4 effector memory cells implicating physical exercise as a pacemaker in lymphocytes [189,190]. While some studies have examined exercise and signaling pathways in muscle [180].

CONCLUSIONS

Like all leukocytes, T cells and macrophages are sensitive to changes in energy supply. The quantity and proportional composition of the substrate supply has both indirect and direct effects on central functions of these cells, including differentiation, activation, cell death, and proliferation. Many intracellular pathways are interrelated between energetic metabolism control and immune cell response. Understanding the regulation and factors influencing the metabolism of immune cells in health and disease can help optimize lifestyle measures for prevention and treatment of inflammatory diseases, and provide insights into healthy aging. Exercise and physical activity clearly has a significant impact on the metabolic status of T cells and macrophages.. It appears that many of the effects that exercise immunology has described over the past 20 years in terms of the effects of physical activity on macrophages and T cells are mediated via a change in the metabolic status of immune cells. It is important now to identify further mechanisms and factors that regulate specific optimization of immune metabolism in health and disease. On this basis it would be possible to optimize exercise, training and physical activity programs based on immunometabolic-regulation, and further develop preventive and sports therapy processes in a forward-looking way.

Author contributions:

JCRN, FSL, JPL and KK conceived the review and drafted the first version of the manuscript. JCRN, FSL and JPL made the figures, reviewed and edited the manuscript. GL, HI, BC, DBP JP and RJS reviewed and edited the manuscript. AMT, HB, BMA, LGM, JCRN, FSL, JPL and KK conceived the review, reviewed and edited the manuscript. All authors approved the final version of the manuscript

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Traumatic Brain Injury Rehabilitation: An Exercise Immunology Perspective

Kannan Singaravelu Jaganathan[1*], Karen A Sullivan[1]

[1] School of Psychology & Counselling, Queensland University of Technology, Brisbane, Australia

ABSTRACT

Traumatic brain injury (TBI) is the largest cause of death and disability globally. The physical and psychosocial consequences after TBI can persist for prolonged periods, and lead to increased health care and economic burden. Exercise has shown promise over recent years as a mode of rehabilitation that alleviates multiple TBI symptoms; but there is a lack of controlled large-scale studies and limited research into the underlying mechanisms. This critical review draws from animal and human studies on exercise immunology to speculate on possible mechanisms that could underlie beneficial outcomes of exercise after TBI. The anti-inflammatory role of exercise, protective role offered by pre-injury exercise, and the need for more objective studies on biomarker analysis are expected to be useful considerations to develop optimal post-TBI exercise rehabilitation programs. Future studies can consider investigating the specific immunological processes induced by exercise in consideration of individual differences and non-aerobic exercise modalities.

Keywords: traumatic brain injury, exercise immunology, neuroinflammation

Corresponding author:
Kannan Singaravelu Jaganathan, School of Psychology and Counselling, Kelvin Grove Campus, Queensland University of Technology, Kelvin Grove, 4059, Queensland Australia.
Email: k.singaravelujaganathan@connect.qut.edu.au

INTRODUCTION

Traumatic Brain Injury Rehabilitation: An Exercise Immunology Perspective

Traumatic brain injury (TBI) is the largest cause of worldwide death and disability around the world. Each year, it is estimated that up to 69 million people will experience a TBI globally (1), leading to healthcare costs and economic burden to patients and caregivers. The consequences of TBI can include a wide range of physical, cognitive, and emotional problems that require personalised care. Many patients take a long time to recover, with a majority never fully returning to pre-injury functioning (2, 3).

Currently, multimodal approaches are favoured to treat post-TBI symptoms. These approaches can include psychological therapy and education on TBI; however, more evidence is needed to identify an approach that can consistently demonstrate positive outcomes. Emerging evidence indicates that an exercise component could be beneficial in such programs because it can influence symptoms across multiple domains, but we lack clear rehabilitation guidelines about critical matters such as the timing, intensity, and frequency of exercise bouts. The underlying mechanisms of exercise that could offer benefits after a TBI are also not clearly understood. It is expected that drawing from literature on exercise immunology could offer some answers. This review will draw from existing literature on TBI pathophysiology and exercise rehabilitation to establish inferences about how exercise could influence post-TBI outcomes. While previous literature has explored these component topics separately, there is value in considering the connections between them. If exercise immunology can account for the apparent benefits of exercise for TBI rehabilitation, this could support program refinements and further development of much needed treatment programs.

Traumatic Brain Injury

Traumatic brain injury (TBI) is defined as “physical damage to, or impairment of brain function, or other evidence of brain pathology, caused by an external force”, usually applied to the head (4). Clinically, the effects on brain structure and function are inferred from any period of loss of consciousness (LOC), post-traumatic amnesia (PTA), any neurological deficits (e.g., weakness, loss of balance, vision problems), or any alteration in mental state (e.g., confusion, disorientation) (4). TBIs are mostly caused by falls, motor-vehicle accidents, and sports-related injuries. While it is common to classify TBI as mild, moderate, and severe based on the duration of key

clinical indicators, such as LOC and PTA (5), these indicators do not always predict the magnitude of clinical and functional outcomes in individuals. Most TBIs are mild injuries (mTBI), also referred to as concussion in the literature (5-7), but the term concussion is less used, because it lacks diagnostic utility (8). In general, TBIs of all severities can cause physical and psychosocial consequences (5), resulting in increased healthcare burden and economic costs. A minority of people with mTBI can also suffer from a range of physical, cognitive, and emotional problems called post-concussion symptoms that can be persistent for months or years (9, 10). A history of mTBIs or sub-concussive injuries over prolonged periods is also thought to increase the risk for neurodegenerative conditions such as chronic traumatic encephalopathy (CTE) and Alzheimer's disease (AD) (8, 11).

TBIs pose considerable prognostic challenges. The diagnosis of moderate or severe TBIs can be aided by techniques such as neuroimaging and injury modelling from animal/human studies due to more measurable tissue damage (8). Along with physical symptoms resulting from primary trauma, the heterogeneity in factors across individuals (e.g., pre-existing psychological problems, history of injury) can result in varying levels of immune system activation and modulation, changing the clinical and functional course of outcomes post-TBI (12). This can plausibly explain why symptomology and recovery trajectories can be different in people after TBI. For those with mTBI, including the small proportion of whom experience persistent post-concussion symptoms, the pathology is less clear, given the lack of overt contusions or haemorrhage (8). The use of different clinical definitions and indicators (13), the lack of sufficient systematic evaluation of the impact of TBIs across the lifespan (14, 15), the fact that people with milder injuries might also require dedicated treatment from healthcare professionals (16) and the absence of reliable prognostic biomarkers especially for milder injuries (17) further affect the understanding of TBI outcomes. Notwithstanding such complexities, a review to better understand how rehabilitation efforts such as exercise work to improve outcomes can provide a neurobiological rationale and advance the field.

CURRENT REHABILITATION FOR TBI

Existing rehabilitation for TBI focuses on interdisciplinary, goal-oriented approaches to reduce disability and improve functioning in people. Neurocognitive therapies have shown promise in identifying and treating attention and memory problems (18, 19). Visual scanning techniques can assist people with visuospatial deficits (20), while more targeted approaches such as physical therapy and vestibular therapy are often used to aid motor recovery, vertigo, or balance problems (21-23). While such individualised approaches are ideal, the heterogeneity in clinical and functional outcomes (24), taken together with the difficulty of reliably predicting prognosis (25) make the provision of comprehensive care challenging and resource intensive. Individualised approaches are expected to address specific clinical outcomes post-TBI, but the underlying mechanisms behind one or more treatment modalities have not been clearly established. Exploring the rationale behind why a mode of rehabilitation might be effective through a neurobiological lens can be useful to make informed and objective decisions for TBI patients.

Exercise has shown promise with TBI rehabilitation in recent years. Studies have demonstrated that exercise can increase cerebral growth factor levels (26-29), reduce apoptosis (30-32), promote neurogenesis, neuronal survival, and regeneration (33-36), reduce lesion size (37, 38) , modulate inflammatory responses (39), reduce astrocytosis (40, 41) , and improve cerebral blood flow (42, 43). Findings from animal studies have contributed towards exercise being considered as a potential non-pharmacological approach to aid recovery after TBI. Given that symptoms from mTBIs are less incapacitating, and there can be a heightened risk of mobility in severe TBI patients, exercise rehabilitation research has largely focused on people with milder injuries (i.e., mTBIs or concussion). Most of the exercise studies have also focused on sports-related concussions (44), as athletes are expected to be more conditioned and less susceptible to adverse risks from exercise post-injury.

The efficacy of exercise rehabilitation for TBI is mixed in the literature. A lack of controlled studies with large samples tempers any general conclusions that exercise could be an effective treatment option. Nonetheless, several reviews on exercise rehabilitation after mTBI have demonstrated moderate support of exercise over and above a natural course of recovery (45-51). Owing to the salutary effects of exercise on positive physical and psychological health outcomes, exercise is often put forward as a non-pharmacological treatment option that can address one or more symptoms across physical, psychological, and cognitive domains after TBI (i.e., 'pan-domain') (52). However, ambiguity remains around exercise variables that are optimal, and that can be replicated in larger studies. As with other TBI rehabilitative modalities, physiological mechanisms underpinning exercise and its effect on symptoms are also not well understood. It is expected that exercise immunology may hold some answers in relation to restoring some of the deficits arising from TBI pathophysiology. While existing exercise literature predominantly focuses on mTBI, the generic pathophysiology after a brain injury can be understood from injuries of all severities (8). If inferences about exercise immunology can be made from currently available exercise studies, it may set forth the further exploration of how exercise affects recovery from more severe TBIs in future.

PATHOPHYSIOLOGY OF TBI

TBI pathophysiology has been investigated using animal models to better understand neuronal death, blood-brain barrier breakdown, oxidative stress, the influence of neurobiological forces that disrupt cell architecture, as well as the role of neuroinflammation (8, 53). Neuroinflammation, which is a key characteristic of the immune system and of interest to this review, is a protective process in central nervous system (CNS) injury (e.g., TBI, stroke); but if this inflammation is too intense and continues for prolonged periods, it can be detrimental, and cause secondary injury after TBI (54). While there is yet to be conclusive evidence on direct causal effects, there is emerging literature that has associated the cascade of processes in secondary injury with cognitive and emotional symptoms post-TBI (8, 55). Secondary injury is also considered a risk factor for neurodegenerative diseases, and there is increasing focus on this in recent literature (8, 11). In TBI,

the primary injury occurs immediately after cerebral trauma resulting in contusion of brain tissue, damage to blood vessels, and axonal shearing, all leading to disruptions in brain function (56-58). Secondary injury can ensue through a complex cascade of neurobiological processes such as ischemic and hypoxic damage, cerebral oedema, elevated intracranial pressure, and infection (56-58). This cascade of neurological and metabolic activity is thought to be induced by an interplay of several factors, including glutamate excitotoxicity, perturbation of cellular calcium homeostasis, membrane depolarisation, mitochondrial dysfunction, inflammation, apoptosis, and diffuse axonal injury (59, 60).

Specific to mTBI, while the general cascade of events is similar to that observed in more severe TBI, increased attention is placed on less observable neurometabolic activity. Immediately after an mTBI, disruption of neuronal membranes, diffuse axonal injury and cellular responses are expected to demand significant energy. In efforts to restore homeostasis, cellular membranes shift into a state of overdrive; but disruptions to cerebral blood flow cause a metabolic crisis (55, 61). This "vulnerability" phase is proposed as a possible explanation for post-concussive vulnerability, persistent symptomology, as well as considerable risks for consecutive injuries (i.e., second impact syndrome).

POST-TBI NEUROINFLAMMATION

In research on post-TBI neuroinflammation, both animal and human studies provide evidence of both central and peripheral inflammatory responses (62-64). These responses include, but are not limited to, activation of resident microglia (e.g., central response) and recruitment of macrophages, dendritic cells, neutrophils, B and T lymphocytes, and meningeal inflammation (e.g., peripheral responses) (8, 57, 65-68). Animal studies have consistently demonstrated an upregulation of proteins such as glial fibrillary acidic protein (GFAP) and ionised calcium-binding adaptor molecule (Iba1), as well as increased levels of proinflammatory cytokines and chemokines after a TBI (66, 67, 69). Exacerbation of neuroinflammation in repeated injuries in quick succession has also been observed (70, 71), lending further support for the potential ramifications of excessive neuroinflammatory processes in such circumstances.

The clinical indicators of moderate/severe TBI can be very different to mTBI, but as with the general pathophysiology, the neuroinflammatory processes that set in after an injury are thought to be similar (8). More importantly, the prolonged state of proinflammatory processes can affect neurobiological recovery because of the influx of infiltrating cells from peripheral systems (72), which in turn influence resident microglia activity and lead to persistent clinical outcomes (73-76). mTBI studies primarily show central inflammatory responses and activation of resident immune cells; but there is no substantial evidence of infiltration of peripheral immune cells, and its role in persistent symptomology. Meningeal inflammation, microglial activation, and some monocyte/macrophage recruitment to the cerebrovasculature has been noted in mild injuries (8). The magnitude and duration of inflammatory responses can be expected to increase in proportion to the severity of the injury and persist for prolonged periods (54, 77-79) — especially in the presence of parenchymal bleeding, haemorrhage or structural injury. A recent study revealed traces of neuroinflammation up to 1 year after an mTBI (80). This suggests that inflammatory processes can persist (even if injuries are mild) and highlights the need for more research into better identification and prediction of such post-TBI neuroinflammatory processes for different injury severities.

To summarise, neuroinflammation in the brain is a key immune response to CNS injury. It involves the activation of resident glia (microglia and astrocytes) and the recruitment of immune cells in the peripheral system (68). In TBI, microglia play a critical role as the first line of defence, producing anti-inflammatory mediators, clearing cellular debris, and orchestrating neurorestorative mechanisms for recovery (81). However, microglia can be a double-edged sword, because excessive amounts for prolonged periods can produce proinflammatory mediators that can exacerbate brain damage, hinder restorative activity and functional recovery (57, 81). Figure 1 summarises the physiological and neuroinflammatory processes that are thought to contribute towards clinical consequences post-TBI.

Studies on neuroinflammation suggest that interventions targeted to modulate inflammation may potentially reduce secondary injury cascades and improve recovery (53, 57). Yet, the clinical efficacy of measures to regulate inflammation after TBI remains questionable, possibly due to the considerable variations in study protocols, individual differences in immune responses, and the heterogeneity of TBI (53, 65). For these reasons, and established evidence that exercise can alter pro-inflammatory processes and also elicit neuroprotective actions, it is a rehabilitation option worth exploring in greater detail (82-85).

Before proceeding to applications of such models to better understand the potential effects of exercise on post-TBI outcomes, it is important to acknowledge that any generalisations being made to humans through predominantly animal models in this area of work need to take a cautionary approach. The closed and open head models used in animal studies to simulate TBI cannot replicate the representation of the injury in humans because of the much higher pathogenetic heterogeneity (25, 53). Injury severity in animal studies is often classified according to how the injury was induced (e.g., fluid percussion injury vs controlled cortical impact injury), rather than by the severity of clinical indicators observed in humans (53). While efforts are made to ensure some uniformity in animal studies, TBI studies in humans need to consider pre-existing factors, concurrent stressors, and individual differences in physiological make-up, that can all affect immune responses (53). Finally, the neuroinflammatory and immune system responses described in this review are specific to further the understanding of exercise immunology after TBIs. For a more comprehensive review of immunological processes post-TBI, see Postolache et al. (53), McKee and Lukens (78) and Simons et al. (11).

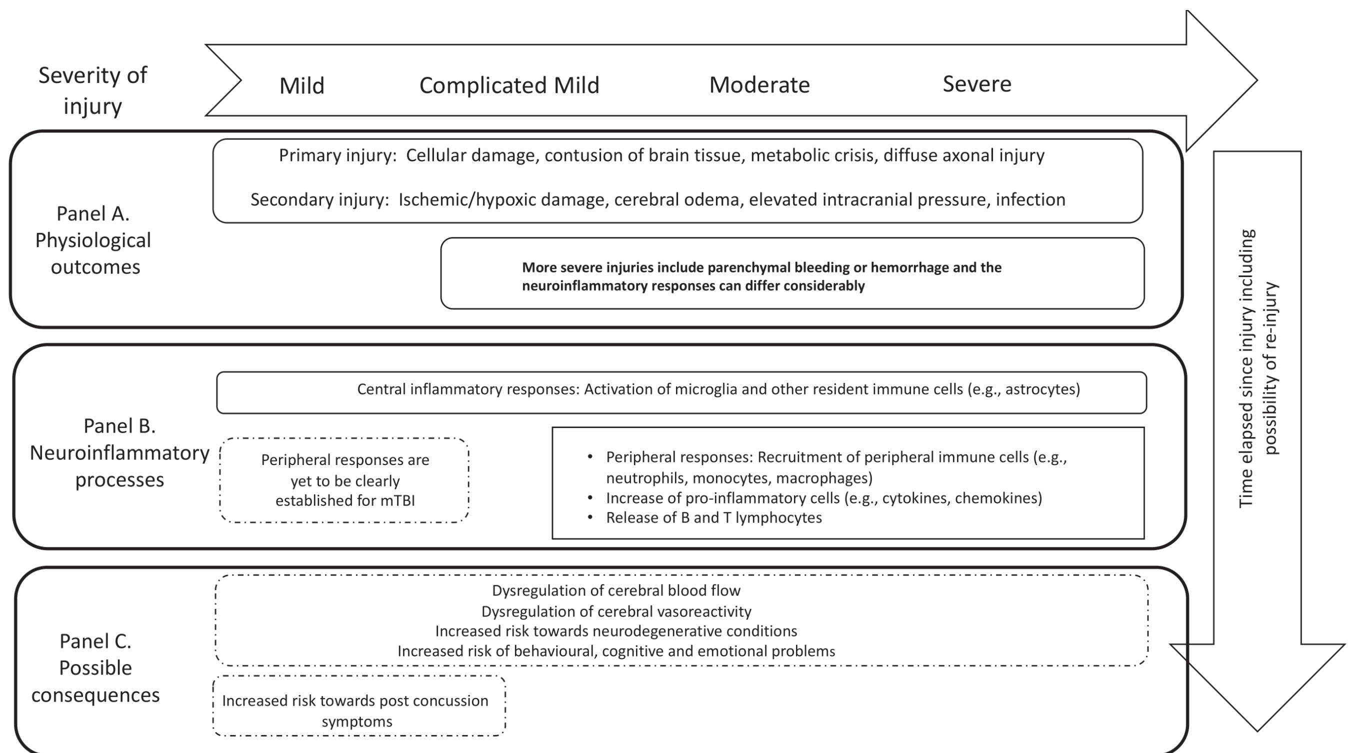

Figure 1. Summary of Physiological and Neuroinflammatory Processes Resulting in Possible Consequences After a TBI From Time of Injury

Note. Panel A briefly describes both the immediate primary and secondary injury outcomes that are part of the neurometabolic cascade of events post-TBI. Panel B outlines the key neuroinflammatory processes that take effect as part of the CNS immune response. Panel C describes some of the possible outcomes and risks that arise due to prolonged neuroinflammation processes. Dotted lines denote the need for further research to establish more conclusive evidence in these areas.

EXERCISE IMMUNOLOGY

Exercise immunology is a relatively new area of study that examines how the immune system responds to exercise (86-88). Progressing from research on acute changes in immune cell function, exercise immunology has now evolved to studying specific clinical outcomes and personalised approaches for various diseases (89). Animal studies on exercise immunology have contributed to the understanding that exercise (i) facilitates endogenous repair mechanisms (90), (ii) elicits neuroprotective actions evidenced in molecular system associated with control of cellular metabolism and synaptic plasticity (91, 92), and (iii) exerts prophylactic effects on acute hyperglycaemia and cerebral inflammatory response induced by TBI (93). Exercise can also alter systematic inflammatory states by reducing pro-inflammatory cytokines and neutrophils in the blood (85, 94, 95). These properties of exercise seem favourable to counteract some of the maladaptive processes after TBI. A closer examination of exercise studies on animals and humans post-TBI follows to draw any useful inferences about exercise immunology.

Animal Studies on Post-TBI exercise

Current animal models examining the effects of exercise have reported positive effects, such as increased levels of neurotrophic factors and increased anti-inflammatory responses. Crane and colleagues (96) compared the effects of voluntary exercise on TBI in rats with bilateral cortical contusions to the medial frontal cortex, or sham surgery. The authors reported greater neuroinflammatory responses in the animals that received the lesion through an increase in GFAB and Iba1 cells, but exercise did not lead to improved cognitive performance. Piao et al. (39) investigated time-dependent effects and the underlying mechanisms of exercise post-TBI using a cortical impact model involving mice. The mice that were exercised 5 weeks after the injury, as opposed to 1-week post-injury, showed significantly reduced memory impairments. Cognitive recovery was associated with attenuation of inflammatory pathways, activation of alternative inflammatory responses, and enhancement of neurogenesis. More importantly, the study concluded that the improved cognitive performance resulting from later exercise could have been due to more optimal balance of microglia expression and increased growth factor levels.

A protective aspect of exercise in the neuroinflammatory process is also a significant consideration. Mota et al. (97) investigated whether previous exercise (i.e., aerobic training) could act as a protective factor post-TBI using a fluid percussion induced (FPI) injury in rats. Rats that underwent 4 weeks of training were compared with sedentary rats after an FPI. The findings suggested that pre-injury exercise could exert protective effects by delaying or preventing secondary cascades post-TBI that led to long-term cell damage and neurobehavioral deficits. An important finding in this study was that regularly performed exercise appeared to have an anti-inflammatory effect, which supports other findings (98, 99). Similarly, de Castro et al. (93) induced neuroinflammation and oxidative stress in rats using an FPI. The study found that prior exercise modulated oxidative-inflammatory functions, putting forward the idea that exercise could be a preventive measure to minimise adverse consequences of TBI.

A series of rodent studies by Griesbach and colleagues (100, 101) further highlighted that voluntary exercise could upregulate brain derived neurotrophic factors (BDNF) and enhance recovery, but only after a period of delay post-injury. It was observed that the time window for resting before exercise could be beneficial increased with the severity of injury (102) and commencing exercising too early impacted growth factors levels and neuroplasticity negatively (103-105). These findings have important implications for exercise rehabilitation and immunology. The later administration of exercise, but not in the acute phase post-TBI, demonstrated benefits in some of these animal studies, suggesting that exercise may be optimal after allowing natural immune responses, including neuroinflammation, to take effect and resolve. Thus, commencing exercise too early post-TBI could disrupt the restorative immunity mechanisms such as cleaning of impacted tissue, protection of infections and increasing brain oxygen demand (95). The optimal time for commencing exercise in humans is yet to be determined, but these animal studies provide some groundwork to expand upon.

Human Studies on Post-TBI exercise

Over the past decade, there has been an increase in studies investigating the effects of exercise on post-TBI outcomes. As pointed out previously, most exercise studies has focused on athletes with mTBI/concussion, in view of the risk and ambulatory concerns for those with more severe TBIs. Early approaches to post-concussion rehabilitation involved prolonged rest or a "cocooning" model of care, emphasising sensory deprivation (106). The effectiveness of such sedentary methods was questioned and promising evidence from animal studies led to controlled studies on exercise in humans. Leddy and colleagues (107) were one of the first research groups to establish the safety of exercise rehabilitation in the post-acute stages after mTBI. Subsequent studies led to the establishment of graded sub-symptom threshold exercise, an aerobic exercise protocol that entailed progressive exercising to an intensity just below that which could trigger symptoms, demonstrating moderate benefits in symptom outcome (108-112). Derivatives of this hallmark feature have been adopted in various exercise studies in post-concussion rehabilitation research (113-117), but participants have been mostly athletes and adolescents, with a focus on post-intervention symptomology.

Most of the human exercise studies have not drawn direct relationships associating mechanisms of exercise immunology, but some parallels can be drawn between exercise immunology literature and post-concussion exercise rehabilitation. Leddy et al. (108) used an fMRI study to demonstrate restored patterns of hemodynamic response to pre-injury levels after an aerobic exercise intervention. Clausen and colleagues (118) pointed out that exercise intolerance after a concussion was attributed to abnormal cerebral blood flow (CBF) regulation as a result of altered sensitivity to carbon dioxide in the brain and/or circulatory system. The hemodynamic responses and restoration of cardiopulmonary processes observed in these studies can all be plausibly explained as a broader outcome of exercise immunology in effect (85, 89). While these studies did not specifically examine the anti-inflammatory effects of exercise, the outcomes could be indicative of potential by-products of the top-down anti-inflammatory mechanisms of exercise. More specifically, the energy crisis after a TBI, the metabolic demands and the neuroinflammatory responses that follow can potentially disrupt neurovascular coupling (i.e., glia, neurons and blood vessels acting as an integrated unit to distribute CBF), cerebral vasoreactivity (i.e., CBF responses to changes in carbon dioxide that is a vital homeostatic function) and cerebral autoregulation (i.e., regulatory mechanisms that counteract effects of arterial pressure fluctuations). The regulatory effects of exercise on such profound immune responses through hemodynamics (87), metabolic and cell function (88) have been noted in both exercise and post-concussion literature (119, 120).

The sub-symptom threshold exercise paradigm, distinctive to post-concussion rehabilitation, and the window of time to allow before exercise commencement in post-concussion literature are other areas that may also be linked with exercise immunology. There is evidence from exercise immunology literature, albeit contentious, that unaccustomed high-intensity exercise (i.e., practised by some elite athletes and the military) can be counterproductive to the immune system (86, 121). The current consensus is for exercising at a moderate intensity to be most conducive for optimal immunology processes to take effect (89). This appears to mirror the advice for post-concussion exercise rehabilitation, which recommends 80-90% of the sub-symptom threshold heart rate (HR) on a systematic assessment of exercise tolerance (i.e., a treadmill test) or 50-60% of the age-predicted maximum HR. Similarly, it is a common recommendation for sports-related concussion rehabilitation to allow an initial period of rest after a concussion before attempting any progressive return to activity. It is possible to draw a link here to previously observed findings in exercise immunology, whereby exercising too soon post-injury was found to impede the natural immune response. Interestingly, some post-concussion exercise studies have administered exercise in very acute stages (i.e., 2-5 days post injury) with encouraging findings (110, 122, 123). While it is difficult to interpret meaningful findings from these studies (given the small sample sizes), a possible explanation could be that mild injuries may not necessitate a prolonged period of natural immune responses as compared to more severe TBIs.

Implication of Findings

Taken together, despite the challenges in translating findings from animal models to humans, the promising findings from exercise studies have established some congruence. Both animal (124) and human studies (125) have demonstrated positive effects of aerobic exercise on cognitive and motor performance after TBI. Studies on TBI patients have also reported improvements in depression and anxiety after post-injury exercise (126, 127). As the exploration of exercise immunology specific to post-TBI rehabilitation can be considered to be in its nascent stages, any inferences drawn are speculative at this stage. Nevertheless, this review aims to integrate findings from both animal and human studies for the specific context of exercise rehabilitation for TBI. The findings have cast some light on the broad and specific mechanisms underlying exercise immunology, the role of prior exercise towards immune responses and the need to consider exercise variables for optimal effects. Figure 2 shows a summary of the possible effects exercise can exert on neurobiological mechanisms to regulate neuroinflammatory responses and potentially improve neurobiological recovery.

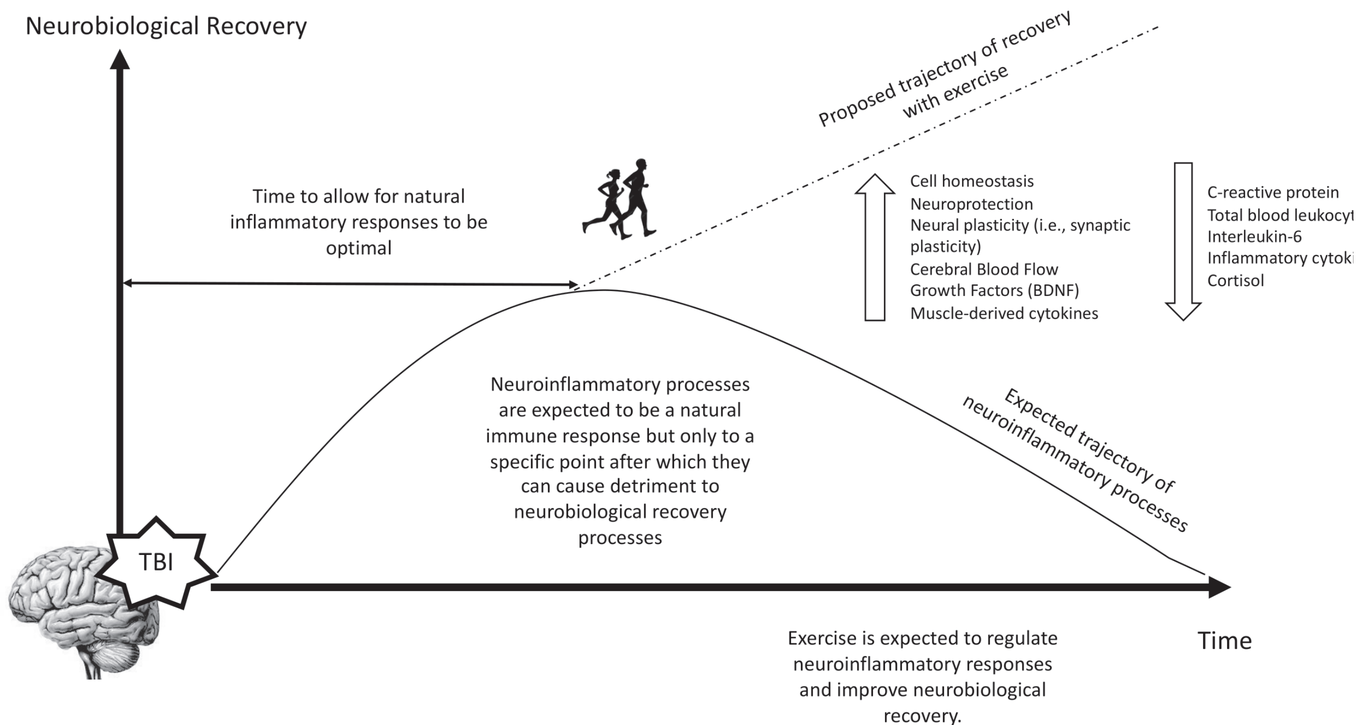

Figure 2. Summary of Proposed Effects of Exercise Modulating Neuroinflammatory Processes Post-TBI
Note. TBI: traumatic brain injury; The figure is a schematic of neuroinflammatory processes that are a key natural immune response to injury in the central nervous system (e.g., traumatic brain injury). The inverted-U curve denotes the dual role of the neuroinflammatory process, where it is beneficial to neurobiological recovery, but only up to a point after which pro-inflammatory processes can be detrimental for recovery. The dotted line indicates the proposed trajectory of recovery that can possibly be exerted through exercise. Exercise is expected to modulate neuroinflammation and facilitate recovery through a wide range of complex neurobiological processes. Some examples of these enhancing (i.e., upward arrow) and attenuating (i.e., downward arrow) processes are included under the dotted line.

FUTURE DIRECTIONS

Future exercise research for post-TBI rehabilitation can consider more specific and objective measures of exercise immunology. There is considerable interest in biomarkers in TBI studies, and exercise studies on TBI patients can examine the various anti- and pro-inflammatory processes resulting from various exercise protocols from start to end of an intervention program. This will significantly enhance TBI rehabilitation efforts to date, allow researchers to better understand immunological processes induced by exercise and perhaps identify exercise prescription guidelines for best effects. Including participants with a history of injury and other pre-existing issues in such studies can also help to clarify differences in the immune responses and the influence of exercise in the different profiles of people at risk of TBI. Notably, the use of biomarkers to identify inflammatory processes is not without problems. For example, some inflammatory cytokines are non-specific to central inflammatory responses, because peripheral injuries can also influence these markers. While cerebrospinal fluid levels may reveal biomarkers more specific to central inflammation, blood-brain barrier dysfunction after TBI can further confound any findings. A potential solution could be to identify both blood and cerebrospinal fluid biomarkers, especially in more severe injuries. For mild injuries, the use of MRI and near-infrared spectroscopy could be useful to determine a more indicative index (57).

More specific studies with larger, diverse samples are required to provide a clearer understanding of how the immune response induced by exercise can be different across demographics such as age and gender. Older age is considered to be a risk factor for TBI (128), and trauma-induced inflammatory processes have shown to increase with age (129, 130). A better understanding of such “immunosenescence” is particularly important, given the increased incidence of TBIs in older people. Exploring how exercise immunology affects this population can help to determine if the benefits of exercise can outweigh any risks for more vulnerable demographics. While the immune response may not be as reactive in older age, further research in this population can help to better understand if there are wider effects of exercise that extend beyond the expected capabilities to modulate inflammatory processes. Gender differences in immune responses also warrant further investigation, considering evidence of neuroprotection from female hormones (131). Most of the animal models studying inflammatory responses post-TBI have been conducted in male animals; how females respond to TBI is still relatively unknown (57). This is particularly important, given that males have an elevated risk of TBI, whereas females have an increased risk of prolonged symptoms after mTBI (7, 132).

The literature on exercise immunology and exercise post-TBI, respectively, has focused on cardiorespiratory exercise (86), and there is a lack of research on other exercise paradigms such as resistance training, or combined aerobic and resistance training. Future studies exploring different exercise paradigms and the potential effects of exercise immunology can be useful to offer more variety in TBI rehabilitation options and could reveal new models of care. There are emerging findings highlighting the potential for resistance training to induce neural adaptations beneficial for synaptic plasticity (133), suggesting that such exercises could be further ex-

plored for those with more severe disabilities after TBI. Resistance training programs that include seated exercise could also be considered to determine if this form of exercise can benefit people with post-TBI ambulatory or vestibular issues, and if such benefits correlate with immunological factors.

While this review emphasised neuroinflammation and the potential anti-inflammatory properties of exercise on post-TBI outcomes, it is important to acknowledge that the benefits of exercise can extend to reduction of apoptosis, increased neurogenesis, enhanced neuroplasticity and increased cerebral blood flow. It is difficult to decouple just the anti-inflammatory characteristics of exercise, as this can be impossible. Any benefits observed could be the outcome of a cascade of neurobiological activity. Additionally, psychological benefits from exercise such as mood improvements can also be a result of complex neurotransmission and hormonal activity, which cannot be entirely explained from an immunological perspective. Nevertheless, the promise of exercise still holds for its ability to potentially influence post-TBI symptoms across multiple domains, and possibly from a profound system-wide process.

CONCLUSIONS

TBI is complex and can affect people from all walks of life. A multi-factorial approach to understand and develop effective rehabilitation is necessary. Exercise has shown promise over recent years as a pan-domain option to alleviate multiple symptoms across physical and psychological domains. However, specific mechanisms underlying the potential benefits of exercise are not well understood. Both animal and human studies on exercise immunology are useful to cast some light on the anti-inflammatory role and the neuroprotective benefits offered by exercise. While post-TBI exercise studies have yet to explore exercise immunology in detail, current findings from post-concussion exercise rehabilitation studies allow some parallels to be drawn. Speculation that exercise immunology is at work to alleviate post-TBI symptoms should serve as a useful springboard for more objective studies in this area.

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The concept for the article was developed by KSJ and KS. The first author led the drafting of all sections. The second author provided advice on the drafts. Both authors approved the final version of the article.

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The effects of exercise on complement system proteins in humans: a systematic scoping review

Daniela Rothschild-Rodriguez[a,1], Adam J. Causer[b,1], Frankie F. Brown[b], Harrison D. Collier-Bain[b], Sally Moore[c], James Murray[c], James E. Turner[b] and John P. Campbell[b*].

[a] School of Biological Sciences, University of Southampton, UK.
[b] Department for Health, University of Bath, UK.
[c] Department of Haematology, Royal United Hospitals Bath NHS Foundation Trust, UK.
[1] Joint first authors.

ABSTRACT

Background: The complement system is comprised of the classical, lectin and alternative pathways that result in the formation of: pro-inflammatory anaphylatoxins; opsonins that label cells for phagocytic removal; and, a membrane attack complex that directly lyses target cells. Complement-dependent cytotoxicity (CDC) – cell lysis triggered by complement protein C1q binding to the Fc region of antibodies bound to target cells – is another effector function of complement and a key mechanism-of-action of several monoclonal antibody therapies. At present, it is not well established how exercise affects complement system proteins in humans. Methods: A systematic search was conducted to identify studies that included original data and investigated the association between soluble complement proteins in the blood of healthy humans, and: 1) an acute bout of exercise; 2) exercise training interventions; or, 3) measurements of habitual physical activity and fitness. Results: 77 studies were eligible for inclusion in this review, which included a total of 10,236 participants, and 40 complement proteins and constituent fragments. Higher levels of exercise training and cardiorespiratory fitness were commonly associated with reduced C3 in blood. Additionally, muscle strength was negatively associated with C1q. Elevated C3a-des-Arg, C4a-des-Arg and C5a, lower C1-inhibitor, and unchanged C3 and C4 were reported immediately post-laboratory based exercise, compared to baseline. Whereas, ultra-endurance running and resistance training increased markers of the alternative (factor B and H), classical (C1s), and leptin (mannose binding lectin) pathways, as well as C3 and C6 family proteins, up to 72-h following exercise. Heterogeneity among studies may be due to discrepancies in blood sampling/handling procedures, analytical techniques, exercise interventions/measurements and fitness of included populations. Conclusions: Increased anaphylatoxins were observed immediately following an acute bout of exercise in a laboratory setting, whereas field-based exercise interventions of a longer duration (e.g. ultra-endurance running) or designed to elicit muscle damage (e.g. resistance training) increased complement proteins for up to 72-h. C3 in blood was mostly reduced by exercise training and associated with increased cardiorespiratory fitness, whereas C1q appeared to be negatively associated to muscle strength. Thus, both acute bouts of exercise and exercise training appear to modulate complement system proteins. Future research is needed to assess the clinical implications of these changes, for example on the efficacy of monoclonal antibody therapies dependent on CDC.

Corresponding author:
Dr. J. P. Campbell, Department for Health, University of Bath, UK, BA2 7AY; Tel: +44 (0)1225 385495; Email: J.Campbell@bath.ac.uk

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INTRODUCTION

The complement system is a fundamental component of the innate immune system, which constitutes more than 40 soluble and membrane-bound proteins, as well as constituent fragments (85, 86). Activation of the complement system is initiated by a cascade of reactions described as the classical, lectin or alternative pathways – all of which result in the cleavage of C3 (~187 kDa; Figure 1) (85). The central complement component C3, primarily of hepatic origin with a half-life of ~72-h in blood plasma (8, 31), is the pre-cursor to the formation of: pro-inflammatory anaphylatoxins (C3a and C5a; ~10 kDa) which enable the chemotaxis and activation of immune cells; opsonins (C3b, iC3b and C3d; 35-180 kDa) that label target cells for phagocytic removal; and, the membrane attack complex (MAC; C5b-9; ~1000 kDa) to elicit direct lysis of target cells (86).

The alternative complement pathway is constitutively activated by the spontaneous hydrolysis of C3 to form C3(H2O). C3(H2O) then binds to factor B (~86 kDa) and D (~27 kDa), which leads to the formation of a fluid-phase C3 convertase, C3bBb (~239 kDa; Figure 1). C3bBb, which is unstable due to spontaneous dissociation and thus has a brief half-life of ~90-s (103), facilitates the tick-over production of C3a and C3b in blood plasma. Under normal physiological conditions, complement activation through the alternative pathway is limited as C3b is only capable of binding to cell surfaces within ~60 nm of C3bBb, due to a short half-life of 60-µs and poor attachment efficiency of ~10% (73). To augment complement activation in response to pathogenic invasion, C3b – generated from carbohydrate-collectin and antigen-antibody interactions of the lectin and classical pathways, respectively – interact with properdin-stabilised C3bBb (37, 49), which induces anaphylatoxin-mediated recruitment of phagocytes and amplifies opsonisation of target cells that lack membrane-bound complement regulatory proteins (70).

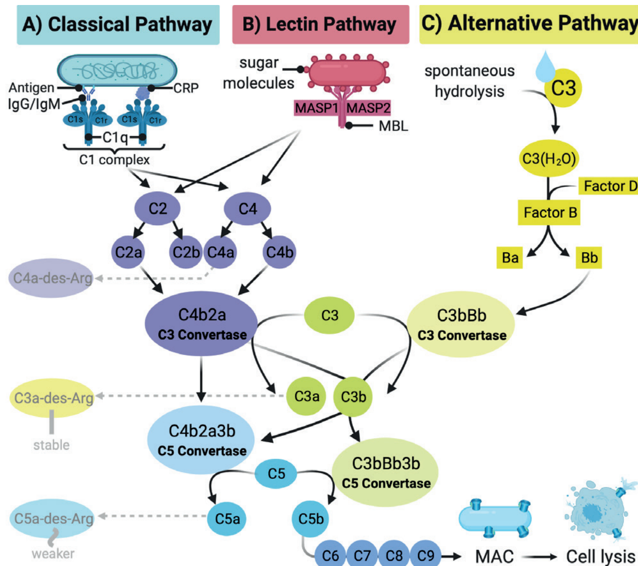

Figure 1. The classical, lectin and alternative pathways involved in the complement cascade. A) Activation of the classical pathway commences when C1q binds the Fc region of target cell bound antibody (either IgG or IgM), causing the serine proteases C1r and C1s to subsequently bind C1q, forming the C1 complex. C-reactive protein can also initiate the classical pathway by binding directly to C1q. C1r and C1s cleave C4 and C2 to form the C3 convertase (C4b2a); B) in the lectin pathway, activating recognition molecules (e.g. mannose-binding lectin [MBL], ficolins and collectins) converge with the classical pathway upon the cleavage of C4 and C2; C) the alternative pathway is initiated by spontaneous hydrolysis of C3 to form C3(H2O), which binds to Factor B and is cleaved into Bb and Ba by Factor D to form a different C3 convertase (C3bBb). Both C3 convertases cleave C3 into C3a and C3b. Complement C3b, is analogous to C3(H2O), and thus is capable of amplifying the alternative pathway; it is also a powerful opsonin that facilitates phagocytosis. With the additional binding of C3b to each of the C3 convertases, C5 convertases are formed (C4b2a3b from the classical and lectin pathways, and C3bBb3b from the alternative pathway). These enzymes cleave C5 into C5a and C5b. C5a is a pro-inflammatory anaphylatoxin along with C3a and C4a, while C5b recruits C6, C7, C8, and C9 to form the membrane attack complex (MAC), where the cascade ultimately results in cell lysis. Anaphylatoxins C3a, C4a and C5a are rapidly converted to C3a-des-Arg, C4a-des-Arg and C5a-des-Arg, respectively, by the cleavage of the arginine residue at the C-terminal. C5a-des-Arg has a weaker pro-inflammatory activity than its whole counterpart, while C3a-des-Arg has no pro-inflammatory activity and does not bind to the C3a receptor. However, C3a-desArg has been found to have additional functions that render it a more stable molecule than its whole counterpart. The function of C4a-des-Arg is not well characterised. This image was created with BioRender.com (Toronto, Canada).

Alternative pathway – as well as classical pathway – activation is also observed during tissue injury, and complement proteins have been implicated in the regeneration of ocular (60), hepatic (78, 133), skeletal (15, 137) and muscular (151, 158) tissues. For instance, rodent studies have demonstrated that the alternative pathway is activated during the early stages (<24-h) of cardiotoxin-induced muscle injury (158); potentially due to the necrotic cell releasing damage-associated markers (e.g. heat shock proteins and high mobility group box-1 proteins) (111, 124). Subsequently, the upregulation of C3a receptor (C3aR)-C3a signalling induces phosphorylation of protein kinase B and NF-κB that exacerbates the transcription of chemokines (e.g. CCL5), which facilitate monocyte trafficking (158). The differentiation of macrophages from an M1-like to M2-like phenotype is responsible for resolving such inflammation by expressing a plethora of anti-inflammatory cytokines (10). M2-like macrophages in muscle tissue are one of the major extra-hepatic sources of complement, which secrete C1q (410-461 kDa) that 'spills over' into circulation and peaks 2- to 4-days following muscle injury (151, 158). As such, interventions that cause muscle damage and inflammation in humans – such as resistance exercise (12), or exposure to unaccustomed, strenuous exercise (106) – could modulate complement activation via an extra-hepatic synthesis of C1q.

Modulating C1q may have wide-ranging clinical implications. Indeed, transient increases in C1q – such as those reported following muscle damage in rodent models (151, 158) – lead to the phagocytic removal and lysis of apoptotic myofibers, thus facilitating skeletal muscle regeneration through classical complement pathway activation, in a process likely mediated by C-Reactive Protein (CRP) and/or natural antibody (146). Moreover, due to its topology of six globular recognition domains and a collagen-like region (85), C1q is capable of binding to over 100 target molecules (61) and thus its secretion facilitates repair and restoration in a host of tissues. Importantly, C1q also binds to the Fc region of immunoglobulin G (IgG) during therapeutic monoclonal antibody treatment of several haematological and solid cancers (28, 59). For instance, rituximab is a chimeric IgG1 monoclonal antibody that targets CD20+ B cells, which is utilised during the treatment of non-Hodgkin's lymphoma and chronic lymphocytic leukaemia (110). Upon binding to CD20, the Fc portion of IgG1 binds the globular region of C1q, whilst the collagen-like region of C1q is bound to C1r and C1s to form the C1 complex on the target cell surface. Following this, the classical pathway is activated and the resultant MAC lyses the malignant cell through complement-dependent cytotoxicity (CDC) (141). As such, increasing the bioavailability of C1q in blood – via interventions that elicit muscle damage and inflammation – could improve the effectiveness of anti-cancer immunotherapies (62, 88).

At present, there is a lack of consensus regarding whether soluble complement proteins are modulated by an acute bout of exercise, exercise training, or in relation to habitual physical activity and fitness. Greater understanding of how exercise affects complement system proteins may yield insight into whether exercise can be harnessed to modulate complement proteins to improve health outcomes, for example during anti-cancer monoclonal antibody immunotherapy treatment. To date, it is well documented that a host of soluble proteins are released in response to exercise – in the presence and absence of muscle damage – including proteins that are directly implicated in complement activation, such as CRP (51, 138, 156). Contrarily, repeated bouts of exercise (i.e. exercise training) and greater fitness levels are associated with reduced complement-activating inflammatory proteins, such as CRP (38). However, the effects of exercise/physical activity on complement proteins themselves are not well understood; in part due to the complexities of complement regulation, as well as the vast number of different complement proteins and constituent fragments involved in the complement cascade.

This situation is complexed further by the likelihood that associations between complement proteins and exercise are likely to be heterogenous dependent upon: timing of the biological sample collection (e.g. during, immediately-post or days/weeks following exercise); the intensity and longevity of exercise; the extent of muscle damage and/or inflammation

caused by exercise; and, whether singular or repeated exercise bouts have been prescribed. As such, a scoping review was employed herein to enable a broad and more exploratory assessment of the effects of exercise on different complement system proteins (134), evaluated on a narrative basis. Whereas, a systematic review specifically assessing the quality of studies and aggregate quantitative data (e.g. meta-analysis) was deemed less suitable (93). The present systematic scoping review aimed to: 1) identify the soluble complement proteins in blood that have been studied in response to an acute bout of exercise, exercise training or in relation to measurements of habitual physical activity and/or fitness; 2) summarise the key findings regarding complement proteins and exercise in healthy humans; and, 3) determine the sources of heterogeneity in the investigation of soluble complement proteins and exercise (e.g. analytical techniques).

METHODS

This review adhered to the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) (136).

Eligibility criteria

Studies were included in the present review if they investigated the association between blood soluble complement proteins and: 1) an acute bout of exercise; 2) exercise training interventions; or, 3) measurements of habitual physical activity and/or fitness. Given that many acute and chronic health conditions affect complement proteins (20), eligible studies recruited healthy human participants. Furthermore, clinical studies were included if they contained a healthy control group who had not undergone an intervention other than those related to exercise. Studies which did not include human participants were excluded due to the substantial inter-species variation in complement function and structure (76, 85). Dissertations, conference abstracts, case studies and review articles, as well as studies which were not available in English or Spanish were excluded.

Literature search

Systematic searches of the PubMed, Embase and Web of Science databases were conducted on Tuesday 20th July 2021. Medical subject headings (MeSH) were searched for within the PubMed database, as follows: ("complement activation"[MeSH] OR "complement system proteins"[MeSH] OR complement[All Fields]) AND (exercise[All Fields] OR physical fitness[All Fields]). No limits on language, date or study type were included. A 'human' filter was used for these systematic searches of the PubMed and Embase databases. Additionally, reference lists from relevant review articles and original research studies that were included in the present review were hand-searched by authors to identify any additional studies.

Study selection

A two-stage screening process was completed independently by two review authors (DRR and AJC). After the removal of duplicates, all titles and abstracts were screened to identify whether the study met the eligibility criteria of the present review, with the exclusion of ineligible references. The full-text articles of the remaining studies were subsequently retrieved and compared to the eligibility criteria. All eligible studies were included in the scoping review (Tables 1-3).

Data extraction and synthesis

Data from selected papers were retrieved independently by two review authors (DRR and AJC). A review-specific form was used to extract information on the studies: design; aim; participants; exercise type, exercise intervention, habitual physical activity or fitness measures; sampling protocol and method of analysis; complement proteins measured; and, key findings. Findings from the systematic literature search were reported by thematic synthesis of the three main themes: acute bouts of exercise ( ), exercise training interventions (Table 2) and cross-sectional studies assessing measurements of habitual physical activity and/or fitness (Table 3).

RESULTS

Selection of studies

A flow diagram of the screening process is presented in Figure 2. Briefly, after removing n = 1065 duplicates and n = 5,374 citations by screening titles and abstracts, n = 157 full-texts were retrieved to determine whether studies were eligible. Of these, n = 66 studies were excluded and a further n = 14 were excluded because the full-text article could not be retrieved. Consequently, n = 77 studies were eligible, and these studies included a total of 10,236 participants and 40 complement proteins and/or constituent fragments.

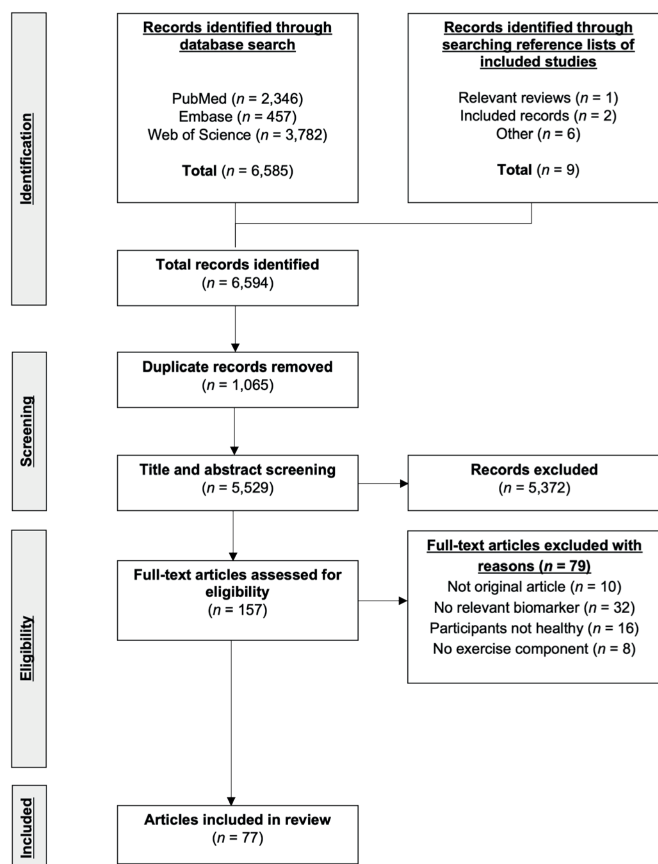

Figure 2. PRISMA flow chart of the study selection process.

Characteristics of studies

A summary of the characteristics of included studies are presented in Figure 3. Briefly, all studies were published between 1981 and 2021, and data were collected from 45 centres in Europe, 21 centres in Asia, five centres in North America, four centres in Africa, one centre in Oceania and one centre in South America. The majority of studies recruited adult participants only (n = 56; 72.7%), whilst paediatric cohorts were studied to a lesser extent (n = 20; 26.3%). Additionally, the majority of studies collected data from only men (n = 35; 46.1%) or a combination of men and women (n = 32; 42.1%); whereas n = 8 (10.4%) studies collected data in women only and n = 2 (2.6%) studies did not report the sex or gender of participants.

Complement proteins were most frequently measured in serum (n = 53; 69.7%) or plasma (n = 23; 29.9%), whilst n = 2 (2.6%) studies did not specify which blood component was analysed. Furthermore, n = 20 (26.3%) studies corrected complement concentrations for changes in plasma volume.

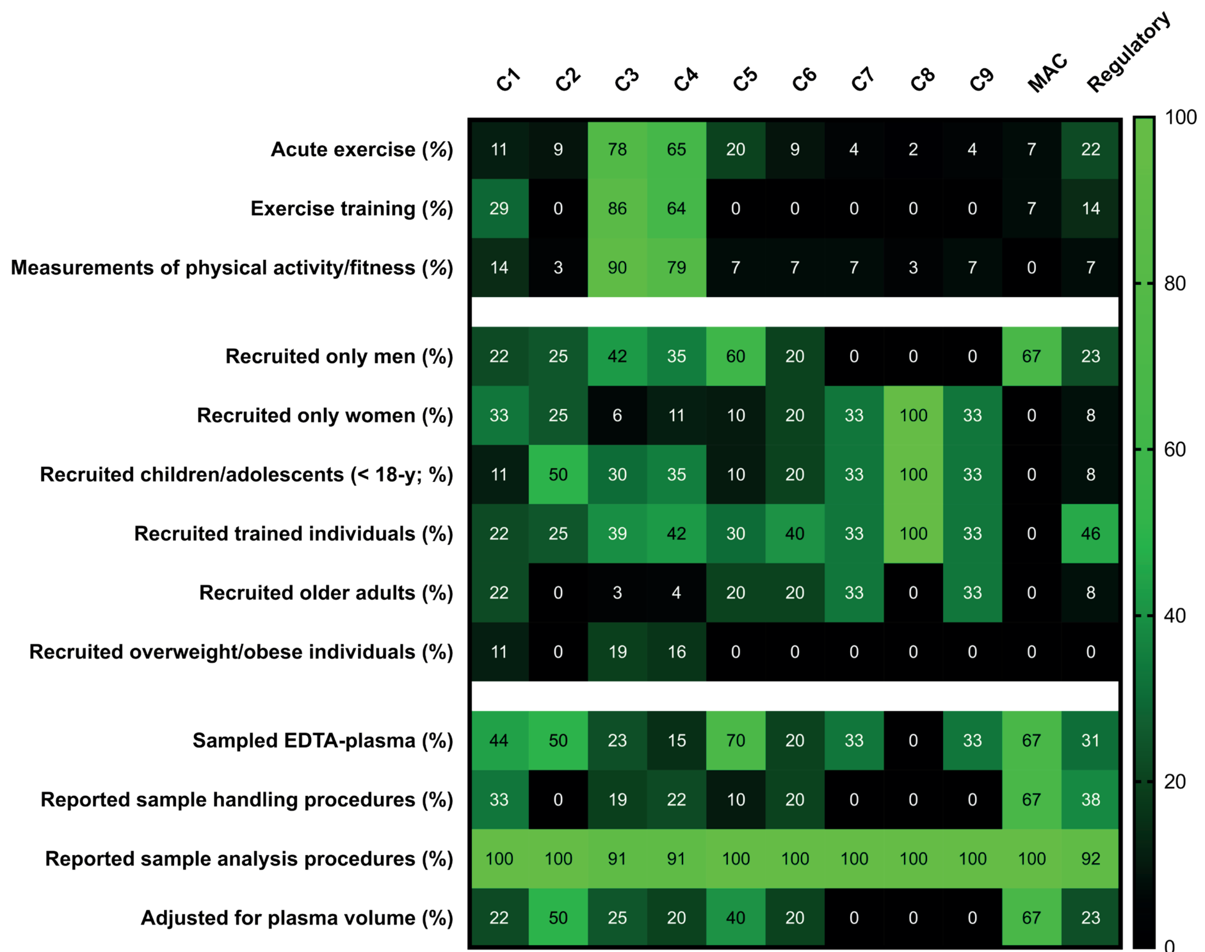

Figure 3. A heat map illustrating the characteristics of included studies. The top compartment demonstrates the percentage of studies that measured each complement protein within each exercise theme (e.g. 11% of studies that investigated the effects of acute exercise measured C1); the middle and bottom compartments demonstrates the percentage of studies that achieved each description within each complement protein (e.g. 22% of the studies that investigated C1 included only male participants).

Characteristics of complement proteins

A total of 40 soluble complement proteins were measured in blood, including five proteins from the classical (C1q, C1s, C1r, C1-inhibitor and C1rs-C1-inhibitor complex), two proteins from the lectin (mannose-binding lectin [MBL] and MBL-associated serine protease-2 [MASP-2]) and two proteins exclusively from the alternative (factor B and properdin) pathways. Convergence proteins of the classical and lectin pathways, the C2 and C4 family proteins (n = 7 proteins), were measured in n = 59 (77.6%) studies. Whereas the convergence proteins of all three pathways, the C3 family proteins (n = 10 proteins), were the topic of n = 64 (84.2%) studies. C5 family proteins were measured in n = 11 (14.5%) studies. Complement proteins that form the MAC (C6, C7, C8 and C9) were the topic of n = 5 (6.8%) studies, and the MAC itself was quantified in a further n = 3 (3.9%) studies. The other soluble proteins investigated were factor I (n = 2; 2.6% studies), factor H (n = 4; 5.3% studies), vitronectin (n = 1; 1.3% study) and clusterin (n = 2; 2.6% studies).

Turbidimetry (n = 23; 30.2%), nephelometry (n = 20; 26.3%) and enzyme-linked immunosorbent assays (ELISA; n = 16; 20.8%) were the most commonly used methods of complement quantification. Less frequently used techniques were radial immunodiffusion (RID; n = 4; 5.3%), radioimmunoassay (RIA; n = 3; 3.9%), mass spectrometry (n = 2; 2.6%), electroimmunoassay (EIA; n = 1; 1.3%) and intermediate gel rocket electrophoresis (n = 1; 1.3%). Notably, n = 6 (7.9%) studies did not specify the methodology for quantifying complement.

Synthesis of results

Acute bouts of exercise

Soluble complement proteins in blood following an acute bout of exercise were the topic of n = 46 (60.5%) studies, and these studies included a total of 879 participants (Table 1). The most common modes of acute exercise were running (n = 21; 45.7%) and cycling (n = 15; 32.6%). Other modes of acute exercise were resistance exercises (n = 6; 13.0%), judo (n = 3; 6.5%) and swimming (n = 1; 2.2%). The duration of acute exercise used in studies ranged from brief (30-s Wingate tests) to multi-staged races (622.2-km over 6-days). The intensity of acute exercise ranged from moderate (e.g. 50% maximal oxygen uptake) to supramaximal (e.g. 'all-out' Wingate tests). Several studies also combined exercise interventions with exposure to heat (n = 2; 4.3%), hypoxia (n = 1; 2.2%) or dehydration (n = 1; 2.2%).

Soluble complement proteins in blood immediately following an acute bout of running exercise were the topic of n = 12 (26.1%) studies. C3 (66, 67, 116, 120), C3a-des-Arg (29), C3c (41), C4 (67, 116, 120), C4a-des-Arg (29) and C5a (17) were elevated; C1q (149), C1r (149), C3 (56, 57) and C4 (56, 57) were lower; and, C1-inhibitor (41, 84, 125), C3 (84, 119, 122), C3d (34), C4 (41, 84, 119, 125), C6 (125) and factor B (41) were unchanged immediately following running, compared to pre-exercise. Soluble complement proteins in blood during the recovery stage (5-mins to 10-h post-exercise) were also the topic of n = 8 (17.4%) studies. C1-inhibitor (125), C2 (65), C3 (97), C3a (65), C4 (65, 97, 125), C4a-des-Arg (29) and C5a (19) were elevated; C3 (65) and C4 (65) were lower; and, C3 (44, 97), C3a-des-Arg (30), C4 (44, 97), C4a-des-Arg (30), C5a (17) and C6 (125) were unchanged during the recovery from running (5-mins to 10-h post-exercise), compared to pre-exercise. C1-inhibitor (125), C3 (44, 56, 57), C4 (44, 56, 57, 125), C5a (19) and C6 (125) were unchanged 12-h to 6-days following the cessation of running, compared to pre-exercise, in n = 5 (10.9%) studies.

Soluble complement proteins in blood immediately following an acute bout of cycling exercise were the topic of n = 13 (28.3%) studies. C3a-des-Arg (18, 30), C4 (24), C4a-des-Arg (30) and C5a (16) were elevated; C3 (56, 57), C4 (56, 57) and C4a (109) were lower; and, C1q (94), C1/C1-inhibitor complex (94), C2 (94), C3 (24, 94), C3a (130), C3a-des-Arg (144), C3c (135), C3d (135), C4 (32, 94), C4a (98, 130), C4a-des-Arg (144), C4b binding protein (94), C4bc (94), C4d (94), C5a (130), C5a-des-Arg (144), MBL (155), MASP-2 (155), factor B (94), factor I (94), vitronectin (94), clusterin (94) and MAC (94) were unchanged immediately following cycling, compared to pre-exercise. Soluble complement proteins in blood during the recovery stage (30-mins to 6-h post-exercise) were the topic of n = 4 (8.7%) studies. C3 (24), C3a-des-Arg (18, 30), C4 (24), C4a-des-Arg (30) and C5a (16) were all unchanged during the recovery from cycling (30-mins to 6-h post-exercise), compared to pre-exercise. Furthermore, n = 2 (4.3%) studies demonstrated that C3 and C4 were unchanged 12- to 24-h following the cessation of cycling, compared to pre-exercise (56, 57). A single study demonstrated that reductions in C1-inhibitor in blood following cycling was only observed in females who were users of oral contraception (50). Additionally, combining cycling with severe normobaric hypoxia (ambient O_2: 12%) increased the transient elevation of C3a-des-Arg, C4a-des-Arg and C5a-des-Arg immediately-post exercise, compared to pre-exercise at sea-level (ambient O_2: 21%) (144).

Soluble complement proteins in blood immediately following ultra-endurance running were the topic of n = 3 (6.5%) studies. A 246-km running race with a mean duration of 32-h and 8-mins, immediately increased C1s subcomponent, C3, factor B and mannose-binding lectin (MBL), compared to pre-exercise (12). Whereas, factor H and the C4-B precursor were unchanged immediately following the 246-km running race, compared to pre-exercise (12). C3 and C4 were unchanged immediately following a 6-day run with an average completed distance of 622.2-km (35). C1-inhibitor, C3 and C4 were unchanged in the days following a 90-km run (126); whereas, C1s subcomponent (12), C3 (12), C6 (126), factor B (12, 126), factor H (12) and MBL (12) were elevated 24- to 72-h following 90-km (126) or 246-km (12) bouts of running exercise.

Soluble complement proteins in blood immediately following a single session of resistance exercise were the topic of n = 2 (4.3%) studies. C3 (101), C3b (101), C3d fragment (101) and clusterin (101) were elevated; and, C1rs-C1-inhibitor complex (114), C3b (114), C3bBbP (114), iC3b (114), C3c (114) and MAC (114) were unchanged immediately following resistance exercise, compared to pre-exercise. C3bc (105) and the MAC (105) were lower, whereas C3 (89) and C5a (55) were unchanged 1- to 96-h following resistance exercise, compared to pre-exercise. Furthermore, C3 (101), C3b (101), C3d fragment (101) and clusterin (101) were elevated 24-h following resistance exercise, compared to pre-exercise. C3 has been reported to increase (22), decrease (91) and remain unchanged (140) immediately following a judo training session; whereas, C4 was unchanged immediately following a judo training session, compared to pre-exercise (22, 91, 140). A single study also reported that C3d remained unchanged following swimming, compared to pre-exercise (33). Mild-to-severe dehydration calculated by changes in plasma volume (22) and an exercise training intervention (not corrected for changes in plasma volume) (91) appeared to blunt the transient post-exercise elevation of C3.

Exercise training interventions

Soluble complement proteins in blood in response to an exercise training intervention were the topic of n = 14 (18.2%) studies, and these studies included a total of 378 participants (Table 2). A training program comprising a combination of steady-state exercise, interval exercise, resistance exercise or judo training were the most studied interventions (n = 4; 28.6%). Other types of exercise interventions were resistance training alone (n = 2; 14.3%), a cross-country skiing, speed skating or volleyball season (n = 2; 14.3%), military training (n = 2; 14.3%), tai chi (n = 1; 7.1%), qigong (n = 1; 7.1%), stair climbing (n = 1; 7.1%) and cycling (n = 1; 7.7%). The duration of each exercise intervention ranged from 17-days to 20-months. Studies most frequently recruited college/university athletes (n = 5; 35.7%) professional athletes (n = 3; 21.4%) or military recruits (n = 2; 14.3%); whilst older adults (n = 1; 7.1%) and less active participants (n = 2; 14.3%) were also studied.

Soluble complement proteins in blood at rest, were investigated immediately following a training program that comprised of more than one mode of exercise by n = 4 (28.6%)

Table 1. Studies investigating the effects of acute exercise on complement proteins in blood.

Reference	Participants	Acute bout of exercise	Complement protein(s)	Key finding(s)
Balfoussia (2014) (12)	8 healthy adult men (median age: 43.2-years [range: 35 to 56-years]).	246-km running event completed in a mean completion time of 32-h and 8-mins.	Plasma C1s subcomponent, C3 precursor, C4-B precursor, factor B, factor H and MBL: venous blood samples were drawn into K_3EDTA tubes pre-, immediately post- and 48-h post-exercise cessation. Blood was centrifuged at room temperature at 1000 ×g for 10-mins and stored at −80°C. Proteins were identified by mass spectrometry. MBL was measured by immune-nephelometric assay.	C1s subcomponent (+323% vs. baseline), C3 (+3% vs. baseline), factor B (+134% vs. baseline) and MBL (+19% vs. baseline) were increased immediately after the completion of the race, whereas factor H was unchanged (+3% vs. baseline) and C4-B precursor was lower (−25% vs. baseline). C1s subcomponent (+300% vs. baseline), C4-B precursor (+30% vs. baseline), factor H (+97% vs. baseline), factor B (+67% vs. baseline) and MBL (+25% vs. baseline) were increased 48-h after the completion of the race, whereas C3 was lower (-47% vs. baseline).
Camus (1994) (16)	11 healthy male students (mean age: 23 ± 2-years).	Static cycle ergometry for 20-mins at a workload corresponding to 80% maximal oxygen uptake.	Plasma C5a: blood samples (n = 6) were collected at pre-exercise, after 10-mins of exercise, immediately post-, 5-mins post-, 10-mins post-, and 20-mins post-exercise. Samples were centrifuged within 5-mins of collection and plasma was stored at −70°C. C5a was identified by ELISA. Plasma C5a was adjusted for changes in plasma volume during and after exercise.	C5a increased after 20-mins of cycling (+76.6% vs. baseline). C5a returned to baseline values within 20-mins of recovery.
Camus (1997) (17)	18 male marathon runners (mean age: 41-years [range: 24 to 64-years]).	Marathon race.	Plasma C5a: blood was collected at rest, between 5 to 15-mins following race completion, and 1-h, and 24-h following race completion. Blood was collected into EDTA containing vacutainers and centrifuged at 2500 ×g for 10-mins. Plasma was stored at −70°C. C5a was identified by ELISA. Plasma C5a was adjusted for changes in plasma volume during and after exercise.	C5a increased immediately after the race (+437% vs. baseline). C5a returned to baseline values within 1-h of recovery.

Cannon (1994) (18)	21 less active participants with normal body mass index (9 women; age range: 20 to 72-years). All participants over the age of 60-years undertook 4-months of dietary fish oil supplementation prior to this study.	3x 15-mins intervals (separated by 5-mins rest) on a recumbent cycle ergometer (n = 5) or downhill (−16% gradient) running on a treadmill at an exercise intensity of 78 ± 6% and 77 ± 2% of maximal heart rate, respectively.	Plasma C3a-des-Arg: blood samples were taken immediately pre-exercise, immediately post-, 4-h post-, 8h post-, 5-d post-, and 12-d post-exercise. C3a-des-Arg was measured by RIA on once thawed samples. Changes in plasma volume during and after exercise were accounted for.	C3a-des-Arg was increased immediately post-exercise (+50.0% vs. baseline) and returned to near-baseline within 4-h. Between group comparisons indicated that increases in C3a-des-Arg with exercise were not attributed to age.
Castell (1996) (19)	18 male runners and 12 male non-exercise controls, who were aged 20- to 40-years (30 men).	The 1991 and 1993 Brussels marathons.	Plasma C5a: Blood samples (n = 4) were collected 30-mins pre-exercise, within 15-mins post-, 1-h post- and 16-h post-exercise. Blood samples were collected into EDTA tubes, the plasma was separated as soon as possible and frozen at −30°C. Plasma C5a was measured using EIA.	In 1991, there was an increase in C5a 5- to 15-mins following the marathon (+358% vs. baseline); however, C5a was not changed from baseline after 16-h (+25% vs. baseline). These results were replicated at the 1993 marathon.
Chishaki (2013) (22)	25 women judoists from a university judo team, were selected due to weight loss after a training session due to loss of body water. Of these, 17 participants experienced mild dehydration (mean age: 20.2 ± 0.7-years); 8 experienced severe dehydration (mean age: 19.8 ± 1.2-years).	2-h and 30-mins of judo training, including: 20-mins warm-up; 30-mins uchikomi; 5-mins rest; 65-mins standing randori; 5-mins rest; 20-mins sitting randori; 5-mins cool-down.	Serum C3 and C4: non-fasted blood samples were collected pre- and immediately post- exercise, and were centrifuged at 3000 rpm for 10-mins and frozen at −30°C. Serum C3 and C4 were both measured by turbidimetry. Both serum C3 and C4 were adjusted for changes in plasma volume during and after exercise.	C3 was increased post exercise (+4.0 ± 4.6% vs. baseline) in the mildly dehydrated cohort; however, there was no change in C3 in the severely dehydrated group (-0.3 ± 1.9% vs. baseline). C4 was not different post-exercise in the moderately (+2.9 ± 6.3% vs. baseline) or severely (-0.6 ± 4.2% vs. baseline) dehydrated groups.
Córdova (2010) (24)	12 male, professional volleyball players (mean age: 25.9 ± 2.6-years).	Incremental (25 W/min) cycling test until exhaustion, at the beginning and the end of a volleyball season.	Serum C3 and C4: fasted blood samples were collected 30-mins before exercise, immediately post-, and 30-mins post-exercise. Samples were analysed by nephelometry. Changes in plasma volume were determined; but it is unclear whether complement proteins were adjusted to account for changes.	At the beginning and end of a volleyball season, incremental exercise increased C4 (+21.3% and 14.1% vs. baseline, respectively), but changes in C3 were not different (+14.4% and 9.5% vs. baseline, respectively). Neither C3 or C4 were different from baseline following 30-mins of recovery.

Dufaux (1989) (29)	8 healthy male students aged between 20- and 28-years. Participants were moderately trained and habitually ran between 10- to 40-km/week.	Prolonged running race (2.5-h) at an average speed of 3.4 ± 0.4 m/s.	Plasma C3a-des-Arg, C4a-des-Arg and C5a-des-Arg: blood samples (n = 8) were collected 1-h pre-, during (1-h), immediately post-, immediately post-, 1-h post-, 3-h post-, 24-h post- and 48-h post-exercise. Blood was drawn into disodium-EDTA tubes and centrifuged at 2000 ×g, 4°C for 15-mins and frozen at −70°C for 2-weeks. C3a-des-Arg, C4a-des-Arg and C5a-des-Arg were measured by RIA and adjusted for changes in plasma volume during and after exercise.	C3a-des-Arg was higher during and immediately after exercise, compared to baseline. C4a-des-Arg was higher during, immediately after and during recovery (1 and 3-h). C5a-des-Arg was below the limit of detection at all time points.
Dufaux (1991) (30)	11 healthy, male, moderately trained students, aged between 19- to 25-years.	Incremental cycling test (10 W/min) until exhaustion.	Plasma C3a-des-Arg and C4a-des-Arg: blood samples (n = 5) were collected 30-mins before, immediately before, immediately post-, 30-mins post- and 60-mins post-exercise. Blood was drawn into disodium-EDTA tubes and centrifuged at 2000 ×g, 4°C for 15-mins and frozen at −70°C for 2 weeks. C3a-des-Arg and C4a-des-Arg were measured by RIA and adjusted for changes in plasma volume during and after exercise.	Both C3a-des-Arg and C4a-des-Arg were higher immediately following exercise (+43.7% and +35.7% vs. baseline, respectively), compared to baseline. However, both C3a-des-Arg and C4a-des-Arg returned to baseline within 30-mins
Ernst (1991) (32)	13 healthy male volunteers (mean age: 28.6 ± 2.0-years).	Incremental (50 W every 3-mins) cycling test using the Bruce protocol	Serum C4: blood samples (n = 4) were collected before (10-mins rest), immediately post-, 24-h post- and 72-h post-exercise. C4 was measured by nephelometry. Serum C4 data pre- and immediately post-exercise were normalised for haematocrit.	C4, normalised for haematocrit, did not increase following incremental exercise (+9.6% vs. baseline).
Espersen (1996) (33)	8 male, elite swimmers (mean age: 20-years [range: 18 to 22-years]) and 10 age- and sex-matched controls (mean age: 27-years [range: 22 to 40-years]).	A 5-km competition swim at 'maximal intensity'.	Plasma C3d: blood samples (n = 4) were collected pre-, immediately post-, 2-h post- and 24-h post-swimming. Blood was drawn into an EDTA-coated tube and plasma was frozen at −80°C within an 1-h of collection. Plasma C3d was measured by ELISA. Complement proteins were adjusted for changes in plasma volume with exercise.	C3d did not change immediately following the 5-km swim in swimmers (−3.4% vs. baseline) or controls (+3.4% vs. baseline). There was also no difference in C3d 1- or 2-h recovery compared to baseline.

Espersen (1991) (34)	11 male, elite or well-conditioned, long to middle distance runners (mean age: 28-years [range: 20- to 42-years]).	A 5-km running race (completion time range: 14.47-18.13-mins).	Plasma C3d: blood samples (*n* = 4) were collected 1-week prior the race, immediately post-, 2-h post- and 24-h post-exercise. Blood was drawn into an EDTA-coated tube and plasma was frozen at −80°C within an 1-h of collection. Plasma C3d was measured by ELISA. Correction for plasma volume did not change the results' significance.	C3d did not change immediately following the 5-km race.
Fallon (2001) (35)	8 healthy participants (7 men, 1 woman; mean age: 47 ± 7-years).	The 1996 Colac 6-day race (mean distance completed: 622.2-km).	Serum C3 and C4: blood samples (*n* = 8) were collected 30-mins pre-exercise, and then daily for the 6-days of the race, and within 15-mins of event completion. Blood was drawn in a seated position to Blood was drawn in a seated position to minimise changes in plasma volume, and samples were analysed within 18-h of collection. Serum C3 and C4 were measured by nephelometry. Serum C3 and C4 were adjusted for changes in plasma volume during and after exercise.	There were no changes in C3 or C4 at any time point during or immediately post-race (−12.1% and 0.0%, respectively) compared to baseline.
Gmünder (1990) (41)	16 runners (3 women; 13 men; median age of 2 groups: 28.0 and 33.0-years).	A 21-km run (run duration: 1-h 15-mins-1-h 50-mins).	Plasma C1-inhibitor, C3c, C4 and factor B: blood samples (*n* = 4) were collected the day before supplement intake began, 27-days after supplement intake at rest, within 2 to 5-mins of completion of the run, and 2-days following the run. Blood was collected into heparin vacutainers, immediately cooled on ice, centrifuged at 3500 ×g for 20-mins, and stored at −70°C.	C1-inhibitor, C4 and factor B were not different during or following the run, compared to baseline. However, C3c was higher after running (median: +13.6% vs. baseline), but not 2 days after the run (median: +3.0% vs. baseline).
Hanson (1981) (44)	6 male runners who trained 9.7-16.1-km/day (aged 27-40-years).	A 12.9-km run on a level pavement at an average velocity of 13-km/h and a work rate corresponding to 72% maximal oxygen uptake.	Plasma/serum C3 and C4: blood samples (*n* = 3) were collected 15-mins pre-exercise, 10-mins and 24-h post-exercise. Blood samples were measured by commercially available kits.	Neither C3 (+10.8% and +15.7%, respectively) or C4 (+3.7% and +3.7%, respectively) increased 10-mins or 24-h post exercise, compared to baseline.

Huisveld (1983) (50)	20 highly trained women cyclists; of which, 10 were (mean age: 25.6 ± 3.4-years) and 10 were not (mean age: 20.4 ± 4.3-years) users of oral contraception.	Stepwise incremental cycling test until exhaustion (mean duration: users of oral contraception, 11.1 ± 1.4 mins; non-users of oral contraception, 11.1 ± 2.7 mins).	Plasma C1-inhibitor: blood samples (n = 2) were collected pre-exercise and immediately post-exercise. Blood was drawn into a plastic tube containing 3.8% sodium citrate. Plasma C1-inhibitor was quantified by EIA and reported as a percentage of the amount present in 1 mL of a normal plasma pool of 40 healthy donors. Pre- and post-exercise data were corrected for haemoconcentration.	C1-inhibitor was lower immediately post-exercise in oral contraceptive users (−6.7% compared to baseline) but was not changed in non-users (−1.7% compared to baseline).
Kanda (2013) (55)	9 untrained healthy men (mean age: 24.8 ±1.3-years).	Calf-raise exercise (10 sets of 40 repetitions, with 3-mins rest between sets) at 0.5 Hz by the load corresponding to 50% body weight.	Plasma C5a: blood samples (n = 7) were collected pre-, 2-h post-, 4-h post-, 24-h post-, 48-h post-, 72-h post-, and 96-h post-exercise. Blood was collected into vacutainers containing EDTA and were centrifuged at 1000 ×g for 10-mins. Plasma was stored at −80°C and analysed by ELISA.	There were no differences in C5a 2-h (−5.3%), 4-h (−15.7%), 24-h (−13.2%), 48-h (−10.5%), 72-h (−7.9%) or 96-h (−7.9%), compared to baseline.
Karacabey (2005) (57)	40 sportswomen who played volleyball 3 times per week for at least 5-years and 20 healthy age-matched less active women.	Either 30-mins of treadmill running at a speed corresponding to 70% predicted maximal oxygen uptake (n = 20) or a 30-s maximal Wingate test (n = 20).	Serum C3 and C4: blood samples (n = 5) were collected pre-exercise, immediately post-, 4-h post-, 48-h post, and 5-days post-exercise. Blood samples were separated as soon as possible and stored at −80°C. C3 and C4 were measured by turbidimetry.	Aerobic exercise resulted in a decrease in C3 and C4 immediately after exercise (−35.4% and −56.0%, respectively), but no differences were observed 4-h (+2.4% and +12.0%, respectively), 48-h (+1.8% and −12.0%, respectively) or 120-h (+1.8% and −16.0%, respectively) after exercise, compared to baseline. Wingate exercise resulted in a decrease in C3 and C4 immediately after exercise (−31.3% and −50.0%, respectively), but no differences were observed 4-h (+1.2% and −4.2%, respectively), 48-h (+0.6% and −8.0%, respectively) or 120-h (−1.2% and −12.5%, respectively) after exercise, compared to baseline.

Karacabey (2005) (56)	40 elite male participants who played volleyball 3 times per week for at least 5-years and 20 healthy age-matched less active male controls.	Either 30-mins of treadmill running at a speed corresponding to 70% predicted maximal oxygen uptake (*n* = 20) or a 30-s maximal Wingate test (*n* = 20).	Serum C3 and C4: blood samples (*n* = 5) were collected pre-exercise, immediately post-, 4-h post-, 48-h post-, and 5-days post-exercise. Blood handling and analytical procedures were not clearly stated by authors.	Aerobic exercise resulted in a decrease in C3 and C4 immediately after exercise (−41.6% and −54.2%, respectively), but no differences were observed 4-h (−5.1% and +4.2%, respectively), 48-h (−2.8% and −8.3%, respectively) or 120-h (−5.1% and −12.5%, respectively) after exercise, compared to baseline. Wingate exercise resulted in a decrease in C3 and C4 immediately after exercise (−39.9% and −61.1%, respectively), but no differences were observed 4-h (−5.2% and −11.1%, respectively), 48-h (−1.2% and −5.6%, respectively) or 120-h (−6.4% and −9.3%, respectively) after exercise, compared to baseline.
Kostrzewa-Nowak (2020) (65)	51 physically active men with a mean age of 16-years (range: 15 to 21-years).	Either an 'aerobic' 20-m shuttle-run test or an 'anaerobic' repeated speed ability test.	Serum C3 and C4, but plasma C2, C3a, iC3b: blood samples (*n* = 3) were collected pre-exercise, within 5-mins of exercise cessaion, and 1-h post-exercise. Blood was collected into S-Monovette tubes for serum separation. Serum C3 and C4 were measured by a colorimetric assay. Plasma C2, C3a and iC3b were measured by ELISA. Changes in plasma volume were calculated by haemoglobin and haematocrit values.	The 'aerobic' exercise decreased C3 (−24.7 to −48.1%) 5-mins post exercise, whereas C3a (+65.4 to +77.3%) and C4 (+5.9 to + 158.9%) were elevated. After 60-mins of recovery from the 'aerobic' exercise, iC3b (+35.7 to +49.2%) and C2 (+11.5 to +18.4%) were increased, compared to pre-exercise. Anaerobic exercise only decreased C2 (−7.5 to −16.6%; during recovery) and C4 (−31.2 to −45.6%; 5-mins post-exercise), but increased C3a (+18.5 to +29.6%; during recovery), compared to pre-exercise.
Kumae (1994) (66)	18 healthy men (mean age: 19.8 ± 1.9-years) who had never participated in any regular exercise training.	A 1500-m run at maximal exertion in a playground, both before and after a 10-weeks of Japanese Military Training (3-4-h of daily training, consisting of: 1-h in the morning; 1-2-h in the afternoon; and, 1-h in the evening).	Serum C3: blood samples (*n* = 2) were collected before and immediately post-exercise. Blood was allowed to clot, centrifuged then stored at −70°C. Serum C3 was measured by nephelometry. Changes in plasma volume were calculated by haemoglobin and haematocrit values.	C3 was higher immediately following the 1500-m run, compared to baseline (+25.0%), both before and after 10-weeks of military training.
Kumae (1987) (67)	26 healthy male students (mean age: 22.6-years [range: 18 to 30-years]).	A 1500-m run in a playground (mean completion time 398.6 ± 42.0 s).	Serum C3 and C4: blood samples (*n* = 2) were collected before and immediately post-exercise. Blood was allowed to clot, centrifuged then stored at −70°C. C3 and C4 were measured by nephelometry.	C3 (+24.3%) and C4 (+16.0%) were higher immediately following the 1500-m run, compared to baseline.

McKune (2009) (84)	15 active, but untrained, men (range: 18 to 22-years).	Downhill running on a treadmill (−13.5% decline) for 60-mins at 75% maximal oxygen uptake.	Serum C1-inhibitor, C3 and C4: blood samples (n = 9) were collected pre-, immediately post-, 3-h post-, 6-h post-, 9-h post-, 12-h post-, 24-h post-, 48-h post- and 72-h post-exercise. Blood was collected into SST-vacutainers and allowed to clot for 30-mins, centrifuged at 2000 ×g for 10-mins and stored at −80°C. C1-inhibitor, C3 and C4 were measured by nephelometry.	C1-inhibitor, C3 and C4 did not change post-exercise, compared to pre-exercise.
Milias (2005) (89)	13 healthy, recreationally active men (mean age: 27.5 ± 3.8-years).	36 (6 sets of 6 repetitions) maximal elbow flexors of the non-dominant arm on a motorised dynamometer. Each repetition was separated by 10-s and each set was separated by 1-mins.	Plasma C3: blood samples (n = 6) were collected immediately pre-, 2-h post-, 24-h post-, 48-h post-, 72-h post-, and 96-h post-exercise. Citrated blood was drawn from the non-exercised arm and stored at room temperature until fibrinogen analysis was performed. Plasma C3 was measured by nephelometry.	C3 was not different from baseline 2-h (+0.8%), 24-h (0.0%), 48-h (−4.2%) or 72-h (−4.2%) after the eccentric exercise intervention; however, C3 was lower 96-h post-exercise compared to baseline (−5.0%).
Miura (2005) (91)	56 male judoists who had practised judo for at least 3-years; but, had not participated in judo exercise for 3-months prior to the study (mean age: 18.0 ± 0.1-years).	A judo session, consisting of: 15-mins preparation; 20-mins of uchikomi; 5x 10-mins sessions of randori; and, a 15-mins cool-down. Participants then enrolled on an intensive 6-month training programme (see Table 2).	Serum C3 and C4: fasted blood samples (n = 2) were collected pre- and immediately post-judo exercise. Blood was allowed to clot for 30-mins at room temperature and centrifuged at 1000 ×g for 10-mins. Samples were stored at −80°C and analysed by nephrometry.	Prior to the 6-month training programme, a single session of judo reduced C3 (−4.2% vs. baseline) immediately following exercise; but, C4 was not different (−4.8% vs. baseline). Furthermore, following 6-months of intensive training, neither C3 (−2.2%) or C4 (−2.3%) were different immediately following a single judo training session compared to baseline.
Mochizuki (1999) (92)	15 athletes (11 cross country skiers; 4 speed skaters; 2 women; 13 men; age range: 16-18-years).	Incremental treadmill test until exhaustion at the following time-points: at baseline prior to the athletic season; during the athletic season; and, after the athletic season (see Table 2).	Serum C3 and C4: blood samples (n = 3) were collected pre-, immediately post-, and 1-h post-exercise. Blood was allowed to clot for 30-mins at room temperature and centrifuged at 1000 ×g for 10-mins. Samples were frozen at −80°C and analysed by nephelometry. Serum C3 and C4 were corrected for changes of plasma volume.	C3 was not different immediately post-exercise before the competitive season (−1.9% vs. baseline); however, C3 was lower immediately post-exercise during (−6.9% vs. baseline) and after (−3.1% vs. baseline) the competitive season. C4 was not different immediately post-exercise before (−1.7% vs. baseline) or after (-0.7% vs. baseline) the competitive season; however, C4 was lower immediately post-exercise during (−7.2% vs. baseline) the competitive season.

Navarro-Sanz (2013) (120)	10 healthy, elite middle-distance runners (5 women; 5 men; mean age: 28.7 ± 4.7-years).	3x 800-m sprints on an open-aired running track at maximal speed, interspersed by 30-s recovery periods.	Serum C3 and C4: blood samples (n = 2) were collected before and immediately after the last sprint. Blood was collected into serum-gel vacutainers and allowed to clot for 30-mins, centrifuged at 4000 rpm and immediately analysed for C3 and C4 by nephelometry.	Both C3 (+42%) and C4 (+29%) were elevated immediately post-exercise, compared to pre-exercise. Strong positive correlations were observed between the increase in C3 and C4 and changes in creatine kinase and post-exercise lactate, respectively.
Nielsen (1995) (94)	7 'normal' controls (6 women; 1 male).	A cycling test at a heart rate of 150 bpm (women) or 170 bpm (male), until they reached a Borg score of 17. This took 10 to 15-mins for women and 15 to 20-mins for men.	Serum C1q, C1-inhibitor, C2, C3, C3a, C3bc, C4, C4bc, C4d, C4b binding protein, C5a, factor I, factor B, vitronectin, clusterin and MAC: blood samples (n = 4) were collected before exercise, immediately after, 2-h and 4-h after the start of cycling. Blood was drawn into a Terumo plastic syringe and analysed as follows; C1q, C2, factor B, factor I, C4d and C4b binding protein by enzyme immunoassays; C3 and C4 by nephelometry. Complement proteins were corrected for changes in plasma volume due to exercise.	C1/C1-inhibitor complexes were not changed by exercise in the normal controls; furthermore, exercise had no effect on any other complement proteins.
Nieman (1989) (97)	11 marathon runners, who had completed at least 3 marathons (11 men; mean age: 42.7 ± 2.1-years) and 9 less active age- and gender-matched controls (mean age: 44.2 ± 1.2-years).	Incremental running exercise until exhaustion.	Serum C3 and C4: fasted blood samples were collected before, during (every 5-mins) and 5-mins, 10-mins, 15-mins, 30-mins, and 45-mins after exercise. Samples were drawn from a catheter and analysed by nephelometry.	Both C3 and C4 were higher 5-mins post-exercise compared to baseline in marathon runners (+15.3% and +11.3%, respectively) and less active controls (+17.1% and +14.2%, respectively). There were no changes in C3 or C4 30-mins post-exercise.
Nijs (2010) (98)	22 healthy and less active women (mean age: 38.9 ± 15.0-years).	Submaximal incremental cycling until achieving 75% age-predicted heart rate maximum; and, self-paced and physiologically limited cycling at a heart rate corresponding to 80% of the anaerobic threshold.	Plasma C4a: blood samples (n = 2) were collected before and immediately after exercise. Blood was collected into EDTA-tubes and analysed by ELISA.	C4a was not changed post-exercise in either submaximal (−13.0%) or self-paced (−11.9%) exercise, compared to pre-exercise.
Oberbach (2011) (101)	15 healthy lean men, aged 20 to 35-years.	60-mins of resistance circuit training at 80% maximal power. The session consisted of 3 repetitions at different stations with 30-s rest.	Serum C3, C3b, C3d fragment and clusterin: fasted blood samples (n = 3) were collected before exercise, immediately after, and 24-h after exercise. Samples were centrifuged at 2500 ×g and 4°C for 10-mins and stored in liquid nitrogen. clusterin and C3 were measured by ELISA.	C3 (+16.3 to 28.9% and +16.3 to 25.6%), C3b (+12.8 to 21.7% and +16.0 to 17.9%), C3d fragment (+19.0% and +21.3%) and clusterin (+23.5% and +12.4%) were 'upregulated' immediately and 24-h post-exercise compared to baseline, respectively.

Paulsen (2005) (105)	11 healthy male students (mean age: 25.7 ± 4.0-years).	300 unilateral, maximal, isokinetic eccentric actions with m. quadriceps. The intervention lasted 35-mins and consisted of 30 sets of 10 repetitions, with 30-s rest between sets.	Plasma C3bc and MAC: blood samples (*n* = 6) were collected 1.5-h before exercise, 1-min, 0.5-h, 1-h, 6-h and 23-h after exercise. Blood was drawn into tubes containing EDTA and centrifuged at 1500 ×g for 15-mins at 4°C. Plasma was then re-centrifuged at 11000 ×g for 5-mins at 4°C and stored at −80°C. Complement proteins were measured by EIA.	C3bc (−21 ± 2%) and MAC (−30 ± 3%) were lower 1-h after exercise compared to baseline; but, returned to baseline within 6-h. C3bc was also lower (−15 ± 2%) 23-h after exercise compared to baseline.
Polli (2019) (109)	22 healthy and less active women (mean age: 38.9 ± 15.0-years).	Submaximal incremental cycling until achieving 75% age-predicted heart rate maximum; and, self-paces and physiologically limited cycling at a heart rate corresponding to 80% of the anaerobic threshold.	Plasma C4a: blood samples (*n* = 2) were collected before and after exercise. Blood was collected into EDTA-tubes, centrifuged at 1500 ×g for 10-mins and stored at −80°C. Samples were analysed by ELISA.	C4a was lower following both submaximal (median change: −17.7%) or self-paced (median change: −8.6%) exercise, compared to pre-exercise samples.
Risøy (2003) (114)	7 men who had performed recreational strength training for at least 2-years (mean age: 26.0 ± 4.0-years).	Squats, front-squats and knee extensions at an intensity of 100% 6-repition maximum (3 sets of 6 repetitions, with 3-mins rest between all exercise and 8-mins rest between squat and front-squat).	Plasma C1rs-C1-inhibitor complex, C3bBnP, C3b, iC3b, C3c and MAC: blood samples (*n* = 9) were collected 30-mins before, 25-mins into, and 5-mins, 20-mins, 35-mins, 50-mins, 65-mins, 5-h and 23-h after exercise. Blood was drawn into vacutainers containing EDTA, placed on ice and centrifuged within 30-mins at 1000 ×g at 4°C for 10-mins. Samples were stored at −20 °C. Enzyme immunoassays were used for analysing complement proteins. Changes in plasma volume were calculated from changes in the plasma total protein concentration.	There was no evidence of systemic complement activation in the hours after heavy strength exercise.
Romeo (2008) (116)	22 healthy men (mean age: 21.27 ± 1.83-years).	A treadmill warm-up for 5-mins at 40% maximal aerobic speed; followed by 60-mins at 60% maximal aerobic speed; and, 5-mins at 30% maximal aerobic speed to cool-down. Laboratory conditions were 35°C and 60% relative humidity.	Serum C3 and C4: fasted blood samples (*n* = 2) were collected immediately before and 10-mins after running. Samples drawn into SST-vacutainers, centrifuged at 3000 rpm at 22-24°C for 15-mins and frozen at −80°C. Complement proteins were measured by immuno-turbidimetry.	C3 (+4%) and C4 (+4.3%) were increased immediately-post exercise, compared to baseline.

Saito (2003) (119)	24 male marathon runners (mean age: 22.9-years) who train an average of 17.4 ± 4.1-h/wk (175 ± 33.8-km).	A 30-km run, which was completed in an average time of 1-h 48-mins and 27-s ± 3-mins 23-s.	Serum C3 and C4: blood samples (n = 2) were collected before and after the run. Blood samples were centrifuged at 2000 ×g for 10-mins and stored at −60°C. Complement C3 was measured by turbidimetry. Data were corrected for changes in haematocrit.	C3 (-0.9%) or C4 (−1.1%) were unchanged immediately post-exercise, compared to baseline.
Sarichter (1995) (121)	36 healthy men aged 21- to 25-years.	70 eccentric contractions (7 sets separate by 3-mins of rest) of the quadricep femoris. Furthermore, a subgroup of participants underwent concentric contractions on a dynamometer one day before and 2, 24, 48, 76, 152, 216-h after eccentric loading.	Serum C3c and C4: blood samples (n = 7) were collected before and 2-h, 1-day, 2-days, 3-days, 6-days, and 9-days after exercise. Blood was collected into serum tubes and measured by turbidimetry.	C3c or C4 remained within the given reference range in all samples from both groups.
Sawka (1989) (122)	5 ‘fit’ male participants who were acclimated to exercising in hot conditions (mean age: 33 ± 2-years).	2 heat-stress tests, which were 120-mins (2 repeated of 15-mins rest and 45-mins exercise on the treadmill) in 35°C and 70% relative humidity. Notably, one test was conducted hypohydrated and one test was conducted euhydrated.	Plasma C3: blood samples (n = 3) were collected at rest and 15-mins, and 40-mins during exercise. Blood samples were collected from a standing position. Changes in plasma volume were calculated by haemoglobin and haematocrit values.	C3 was not altered by exercise; however, hypohydration resulted in a greater C3 compared to euhydration.
Semple (2004) (126)	11 experienced ultramarathon athletes (6 men; 5 women; mean age: 43 ± 9-years).	A 90-km running ultramarathon.	Serum C1-inhibitor, C3, C4, C6 and factor B: blood samples (n = 5) were collected 24-h before their predicted finishing time, immediately after the run and 3-h, 24-h, and 72-h after the run. Blood was allowed to clot for 15-mins, centrifuged at 1000 ×g for 10-mins and stored at −80°C. C1-inhibitor, C3 and C4 were measured by nephelometry. C6 and factor B were measured by RID. Complement protein concentrations were corrected for changes in plasma volume due to exercise.	C3, C4 and C1-inihibitor showed no differences up to 72-h post-ultramarathon. C6 was elevated at 24-h post ultramarathon (+7.8% vs. baseline) and remained elevated at 72-h post. Factor B was elevated at 72-h post-ultramarathon (+12.8% vs. baseline) only.

Semple (2011) (125)	11 active, but untrained, healthy men (mean age: 19.7 ± 0.4-years).	2 bouts of downhill running (−13.5% decline) on a treadmill for 60-mins at 75% maximal oxygen uptake, spaced 14-days apart.	Serum C1-inhibitor, C4 and C6: blood samples ($n = 20$) were collected at baseline, immediately after exercise and 1-h, 2-h, 3-h, 4-h, 5-h, 6-h, 7-h, 8-h, 9-h, 10-h, 11-h, 12-h, 24-h, 48-h, 72-h, 96-h, 120-h, and 144-h after exercise. Blood was allowed to clot for 30-mins, centrifuged at 1000 ×g for 20-mins and stored at −70°C. C1-inhibitor and C4 were measured by nephelometry. C6 was measured by RID.	C1-inhibitor was elevated 9- and 10-h post-exercise, compared to pre-exercise, with concentrations being up to 7% higher during the second exercise bout. C1-inhibitor was back to pre-exercise levels 24-h post-exercise. C4 was elevated 7- and 9-h post-exercise, compared to pre-exercise, with concentrations being up to 17% higher during the second exercise bout. C4 was back to pre-exercise levels 24-h post-exercise. Post-exercise C6 was not different from pre-exercise at any time point, but were up to 4% higher during the second exercise bout.
Sorensen (2003) (130)	29 healthy controls, who were sedentary to moderately active (women:male ratio: 2.5:1; 18 to 45-years).	A submaximal, steady-state cycling for 20-mins at 70% predicted workload.	Plasma C1-inhibitor, C1q, C1r, C1s, C2, C3, C4, C5, C6, C7, C9, factor B, factor H, factor I, C3a-des-Arg, C4a-des-Arg and C5a-des-Arg: blood samples ($n = 5$) were collected immediately before, immediately after and 1-h, 6-h, and 24-h after exercise. Blood was collected into EDTA-containing tubes were centrifuged at 4°C and store at −70°C. Plasma C1-inhibitor, C1q, C1r, C1s, C2, C3, C4, C5, C6, C7, C9, factor B, factor H and factor I were measured by radioimmunodiffusion. Plasma C3a-des-Arg, C4a-des-Arg and C5a-des-Arg were measured by ELISA.	In the control group, exercise did not alter C3a, C4a or C5a at any time-point. Authors did not report the effect of exercise on C1-inhibitor, C1q, C1r, C1s, C2, C3, C4, C5, C6, C7, C9, factor B, factor H and factor I.
Thomsen (1992) (135)	14 healthy, untrained male students, aged 20 to 29-years.	60-mins of cycling at a work rate corresponding to ~75% maximal oxygen uptake.	Plasma C3c and C3d: blood samples ($n = 4$) were collected before exercise, during exercise and 2-h, and 24-h after exercise. Blood was collected into EDTA-tubes and stored at −80°C within 2-h of collection. C3 split products were measured by intermediate gel rocket immunoelectrophoresis.	Cycling did not induce changes in C3c or C3d.

Umeda (2008) (140)	22 male university judoists (mean age: 19.1 ± 0.8-years).	A 2-h judo session (consisting of: 15-mins warm-up; 20-mins of uchikomi; 70-mins of randori; and, 15-mins cool-down), in 28.2 ± 0.4°C.	Serum C3 and C4: fasted blood samples (*n* = 2) were collected immediately before and immediately after the judo session. Blood samples were drawn from a supine position, centrifuged at 3000 rpm for 10-mins and stored at −30°C. Complement proteins were measured by turbidimetry. Post-exercise complement concentrations were corrected for changes in plasma volume.	Compared to baseline, there were no changes in C3 (+1.7%) or C4 (+1.3%) immediately after 2-h of judo training.
Wang (2009) (144)	15 healthy sedentary men (mean age: 23.7 ± 1.5-years).	Cycling at an incremental workload until exhaustion (strenuous) or a work rate corresponding to 50% maximal oxygen uptake (moderate) at several levels of graded normobaric hypoxia with O_2 concentrations of 12% (~4460-m), 15% (2733-m) and 21% (sea-level).	Plasma C3a-des-Arg, C4a-des-Arg and C5a-des-Arg: blood samples (*n* = 3) were collected before, immediately after and 2-h after exercise. Blood samples were collected into EDTA-polypropylene tubes, centrifuged at 10000 ×g at 4°C for 30-mins. Complement split products were measured by ELISA.	Moderate exercise has no effect on complement cleavage products in any of the hypoxic conditions. C3a-des-Arg, C4a-des-Arg and C5a-des-Arg were elevated 2-h after strenuous exercise, but not immediately-post exercise. The combination of severe hypoxia and strenuous exercise were the only conditions to increase C3a-des-Arg, C4a-des-Arg and C5a-des-Arg immediately-post exercise.
Wolach (1998) (149)	Participants were prepubertal girls that were elite gymnasts (*n* = 7) and untrained controls (*n* = 6), aged 10- to 12-years.	20-mins of treadmill running at a heart rate of 170-180 beats per minute.	Serum C1q, C1r, C1s, C2, C3, C4, C5, C6, C7, C8, C9, factor B and properdin: fasted heparinized blood samples (*n* = 3) were collected before, immediately after, and 24-h after the run. Samples allowed to clot at room temperature for 30-mins, centrifuged at 4°C and stored at −80°C. Complement proteins were measured by RID.	C1q (trained: −5.2%; untrained: −6.4%) and C1r (trained: −3.0%; untrained: −3.5%) were reduced immediately-post exercise; whereas, C4 was elevated in untrained participants only immediately post-exercise (+9.9%). C1q (trained: −11.2%; untrained: −9.2%), C1r (trained: −4.0%; untrained: −2.3%) and C7 (untrained: −6.2%) were lower 24-h post-exercise compared to baseline; whereas C4 was elevated (+4.9%) 24-h post exercise in untrained participants only. Other complement proteins were not different between time-points.
Ytting (2007) (155)	14 healthy adults (10 women; 4 men; median age: 49-years [range: 35- to 64-years]).	An incremental cycling test until participants reached 70% to 80% predicted heart rate maximum. The total cycling time was 25-mins.	Serum MBL and MASP-2: blood samples (*n* = 4) were collected before exercise, immediately after and 1-h, and 3-h after exercise. Blood samples were collected in endotoxin-free siliconized glass tubes. Samples were allowed to clot at room temperature for 1-h, centrifuged at 2500 ×g at 4°C for 10-mins and stored at −80°C. Samples were analysed by time-resolved immune-fluorometric assays.	No changes in MBL or MASP-2 were detected between the pre- and post-exercising samples.

Table 2. Studies investigating the effects of exercise training interventions on complement proteins.

Reference	Participants	Exercise intervention	Complement protein(s)	Key finding(s)
Chen (2021) (21)	30 obese, sedentary women (mean age: 65.4 ± 6.6-years).	Either ascending (*n* = 15) or descending (*n* = 15) stair walking training in a 10-story building (110 stairs = 1 repetition) two times a week for 12 weeks. Exercise volume was gradually increased by doubling the number of repetitions every 2-weeks.	Plasma C1q: blood samples taken at baseline and after the 12-week intervention were drawn into EDTA vacutainers. Samples were centrifuged at 2000 rpm for 10-mins and plasma stored at -80°C until further analysis by ELISA.	After the 12-week intervention, plasma C1q concentrations decreased by 51% in the descending stair walking group, compared to baseline. No differences in C1q were seen in the ascending stair walking group.
Córdova (2010) (24)	12 professional volleyball players (mean age: 25.9 ± 2.6-years).	An incremental (25 W/min) cycling test until exhaustion. Samples were taken at the start of and following the 4-month professional volleyball season.	Serum C3 and C4: fasted blood samples were collected 30-mins before exercise, immediately after, and 30-mins after exercise. Protein concentrations were measured by nephelometry. Plasma volume changed were measured; but it was unclear whether complement proteins were corrected.	Resting (+1.7% and +11.3% vs. pre-season, respectively), immediately-post incremental exercise (−2.7% and +4.7% vs. pre-season, respectively) and 30-mins recovery (−12.5% and +1.9% vs. pre-season, respectively) C3 and C4 were not different at the beginning of a volleyball season compared to the end of a 4-month season.
Jia (2016) (53)	60 Chinese male military recruits (mean age: 18.8 ± 1.7-years).	3-months of basic military training.	Serum C3 and C4: fasted blood samples were collected immediately after each military training session, drawn into procoagulation tubes, allowed to clot for 30-mins at 37°C and then centrifuged at 1760 ×g for 15-mins at 37°C. Samples were stored at −20°C and analysed by nephelometry.	Neither C3 (-0.9% vs. baseline) or C4 (+3.8% vs. baseline) were different following 3-months of basic military training.
Kumae (1994) (66)	18 healthy men (mean age: 19.8 ± 1.9-years) who had never participated in any regular exercise training.	1500-m run at a maximal exertion, both before and after a 10-weeks of Japanese Military Training (3-4-h of daily training, consisting of: 1-h in the morning; 1-2-h in the afternoon; and, 1-h in the evening).	Serum C3: blood samples were collected before and immediately after the exercise. This was done both at the start of the training period and after. Blood was allowed to clot, centrifuged then stored at −70°C. Serum C3 was measured by nephelometry. Complement proteins were adjusted for changes in plasma volume with exercise.	C3 was reduced following 10-weeks of military training (−23.5%).
Manzaneque (2004) (77)	29 healthy psychology students, aged 18 to 21-years (14 men; 15 women), were allocate to a control (*n* = 13) or experimental (*n* = 16) group.	Qigong training for 30-mins per day for 1 month. Each qigong sequence contained 8 movements, which were repeated 8 times, equating 64 movements per session.	Serum C3 and C4: blood samples were collected before the training began, and 1-month after the qigong training. Blood samples were collected into a vacutainer tube and centrifuged at 3500 rpm. Complement proteins were measured by nephelometry.	After 1-month of qigong training, C3 was lower in the experimental group compared to the control group (98.89 vs. 109.74 mg/dL, respectively); however, there was no difference in C4 (16.51 vs. 18.02 mg/dL, respectively).

Mashiko (2004) (83)	25 college male rugby players (mean age: 20.2-years).	A training camp for 6-h/day (3-h in the morning and 3-h in the afternoon) for 17-days.	Serum C3 and C4: fasted blood samples were collected immediately before the start of the training camp, and immediately after training camp completion. Samples were centrifuged at 3000 ×g for 15-mins and stored at −80°C. Complement proteins were measured by turbidimetry.	C4 (+22.5%) was increased immediately following the 17-day training camp; whereas, C3 was unchanged from baseline (+1.0%).
Miura (2005) (91)	56 male judoists who had practised judo for at least 3-years; but, had not participated in judo exercise for 3-months prior to the study (mean age: 18.0 ± 0.1-years).	A 6-month training programme, consisting of: 2x interval sprint running sessions per week; 2x resistance exercise sessions per week; 2x 30-mins runs with intermittent sprints per week; 6x judo training sessions per week; and, 1x rest day per week.	Serum C3 and C4: fasted blood samples were collected before and after the training period, both taken immediately following the judo exercise. Blood samples were allowed to clot for 30-mins at room temperature and centrifuged at 1000 ×g for 10-mins. Samples were stored at −80°C until being analysed by nephelometry.	Neither resting C3 (−3.5%) or C4 (−6.2%) were different pre- and post-6 months of intensive judo training.
Mochizuki (1999) (92)	15 athletes (11 cross country skiers; 4 speed skaters; 2 women; 13 male; age range: 16 to 18-years).	Participants underwent: *a)* athletic pre-season, characterised by extensive endurance training and leg-power training; *b)* during the season, characterized by similar training as pre-season to maintain fitness but with additional technical training; and, *c)* post-season, characterised by one months of rest following the final event of the season.	Serum C3 and C4: blood samples were collected before, immediately after, and 1-h after the test during pre-season, peri-season, and post-season. Blood was allowed to clot for 30-mins at room temperature and centrifuged at 1000 ×g for 10-mins. Samples were frozen at −80°C and analysed by nephelometry. Serum C3 and C4 were corrected for changes of plasma volume.	C3 was not different between the pre-season (103 ± 13 mg/dL) and during-season (101 ± 14 mg/dL) measurements; however, C3 was lower following 1-month of rest (−5.8%). C4 was lower during the season (−16.0%) and after 1-months rest (−20.4%), compared to pre-season.
Risøy (2003) (114)	17 male students who had performed recreational strength training for at least 2-years (mean age: 25.9-years).	4-weeks of normal resistance training, followed by an additional 2-week period of heavy leg extensor training in a subgroup of participants ($n = 10$).	Plasma C1rs-C1-inhibitor complex, C3bBbP, C3b, iC3b, C3c and terminal complement complex: blood samples were collected 30-mins before, after 25-mins of exercise and 5-mins, 20-mins, 35-mins, 50-mins, 65-mins, 5-h and 23-h after each exercise session. Blood was drawn into vacutainers containing EDTA, placed on ice and centrifuged within 30-mins at 1000 ×g at 4°C for 10-mins, samples were stored at −20°C. Complement proteins were corrected for changes of plasma volume.	There was no evidence of systemic complement activation after the resistance training intervention.

Semple (2006) (127)	17 professional cyclists (mean age: 28 ± 1-years).	The Vuelta a Espańa, which is a 2956-km cycling race that is conducted over 21 consecutive days.	Serum C1-inhibitor, C3 and C4: fasted blood samples were collected before the race began (0-km), on the first rest day (194-km), and at 164-km. Blood samples were allowed to clot in serum separator tubes at room temperature, centrifuged at 2000 ×g at 4°C for 20-mins and stored at −80°C. C3 and C4 were measured by nephelometery.	C3 and C1-inhibitor were not different after stage 10 (−1.2% and +2.5%, respectively) or stage 19 (+1.2% and +1.4%, respectively); whereas, C4 was elevated after stage 10 (+12.5%), but not stage 19 (0.0%), compared to baseline.
Umeda (2004) (139)	49 male college judoists, who had not participated in judo for 14-days prior to the intervention.	A 20-month training programme, consisting of: 2x interval sprint running sessions per week; 2x resistance exercise sessions per week; 2x 30-mins runs with intermittent sprints per week; 6x judo training sessions per week; and, 1x rest day per week. 38 athletes combined the training camp with calorie restriction.	Serum C3 and C4: fasted blood samples were collected 20-days, 4-days, and 1-day before the competition, and 7-days after the competition. Samples were centrifuged at 3000 rpm for 15-mins and stored at −30°C. C3 and C4 were measured by turbidimetry.	Neither C3 or C4 were altered by pre-competition judo training for 16 (−2.0% and +5.8%, respectively) or 19 (−1.5% and +1.4%, respectively) days. However, complement C3 (−8.5%), but not C4 (−6.2%), was lower 7-days post-competition. Calorie restriction did not affect the response of C3 or C4 to exercise in judoists.
Watanabe (2015) (145)	11 men aged 60- to 81-years.	Resistance training 3 times per week, on alternate days, for 12-weeks. The starting weight used during exercise was 70% of the participants' 1-repition maximum for 3 sets of 10 repetitions using leg curl and extension machines. The rest period between sets was 3-mins.	Serum C1q: fasted blood samples were collected at least 48-h after exercise, both at the beginning and at the end of the study period. Samples were centrifuged immediately at 1500 ×g at 4°C for 15-mins and stored at −80°C. C1q was measured by ELISA.	C1q was reduced (−44.5%) by the resistance training intervention, compared to baseline. The reduction in C1q was associated with an increased thigh cross-section area following resistance training.
Yaegaki (2007) (152)	16 women university judoists, who had rested for 2-weeks prior to the intervention.	A 20-day training programme, consisting of: 2x interval sprint running sessions per week; 2x resistance exercise sessions per week; 2x 30-mins runs with intermittent sprints per week; 6x judo training sessions per week; and, 1x rest day per week. 8 athletes combined the training camp with weight reduction.	Serum C3 and C4: blood samples were collected on the first and last days of a 20-day training period, before the judo competition. Blood samples were measured by nephelometry.	Neither C3 (−12.6%) or C4 (−3.3%) were altered by 20-weeks of intensive judo training.
Yang (2010) (153)	23 healthy adult participants (11 women; 12 men; mean age: 52.1 ± 2.2-years).	12 weeks of tai chi chuan exercise, which consisted of 37 standardised movements that lasted 60-mins (including a 10-mins warm-up and 10-mins cool-down).	Serum C1-inhibitor, C1r subcomponent precursor, C3 precursor, factor H and factor B: blood samples were collected before and after the intervention. Serum was analysed by mass spectroscopy ($n = 3$) and validated by western blot ($n = 20$)	Factor H and C3 precursor were upregulated, whereas factor B, C1r subcomponent precursor and protease C1-inhibitor were downregulated, compared to baseline.

Table 3. Studies investigating the relationship between measurements of habitual physical activity and fitness, and complement proteins in blood.

Reference	Participants	Measurement of physical activity or fitness	Complement protein(s)	Key finding(s)
Agostinis-Sobrinho (2020) (1), LabMed Physical Activity Study	406 healthy adolescents (212 girls, 194 boys, mean age 14.4 ± 1.7-years).	Cardiorespiratory fitness by a 20-m shuttle run test.	Serum C3 and C4: Fasted blood samples were stored for up to 4-h at 4-8°C and analysed by turbidimetry.	There were no statistical analyses conducted that determined the association between cardiorespiratory fitness and complement proteins.
Agostinis-Sobrinho (2018) (3), LabMed Physical Activity Study	529 healthy adolescents (267 girls, 262 boys, mean age 14.3 ± 1.7-years).	Upper body isometric strength by hand-grip dynamometry; lower body explosive strength by a standing long-jump test; cardiorespiratory fitness by a 20-m shuttle run test.	Serum C3 and C4: Fasted blood samples were stored for up to 4-h at 4-8°C and analysed by turbidimetry.	There was no relationship found between fitness and C3 or C4.
Agostinis-Sobrinho (2018) (2), LabMed Physical Activity Study	529 adolescents (267 girls, 262 boys, mean age 14.3 ± 1.7-years).	Cardiorespiratory fitness by a 20-m shuttle run test.	Serum C3 and C4: Fasted blood samples were stored for up to 4-h at 4-8°C and analysed by turbidimetry.	There were no statistical analyses conducted that determined the association between cardiorespiratory fitness and complement proteins.
Agostinis-Sobrinho (2017) (4), LabMed Physical Activity Study	529 adolescents (267 girls, 262 boys, mean age 14.3 ± 1.7-years).	Cardiorespiratory fitness by a 20-m shuttle run test.	Serum C3 and C4: Fasted blood samples were stored for up to 4-h at 4-8°C and analysed by turbidimetry.	The 'fit' group had lower C3 and C4, compared to the 'unfit' group.
Almeida-de-Souza (2018) (7), LabMed Physical Activity Study	412 adolescents (216 girls, 196 boys, mean age 14.9-years [range: 12.6 to 15.7-years]). Notably, 7.5% of the studied cohort were obese and 22.1% were overweight.	Physical activity was assessed by accelerometery.	Serum C3 and C4: Fasted blood samples were stored for up to 4-h at 4-8°C and analysed by turbidimetry.	There were no statistical analyses conducted that determined the association between physical activity and complement proteins.
Almeida-de-Souza (2018) (6), LabMed Physical Activity Study	329 adolescents (184 girls, 145 boys, mean age 15.0-years [range: 13.0 to 16.0-years]). Notably, 7.9% of the studied cohort were obese and 22.8% were overweight.	Physical activity was assessed by accelerometery.	Serum C3 and C4: Fasted blood samples were stored for up to 4-h at 4-8°C and analysed by turbidimetry.	There were no statistical analyses conducted that determined the association between physical activity and complement proteins.
Artero (2014) (11), Healthy Lifestyle in Europe by Nutrition in Adoles- cence (HELENA) Study	639 adolescents (343 girls, 296 boys, mean age 14.9 ± 1.2-years). Of which, 0.8% of the studied cohort were obese and 2.3% were overweight.	Upper body isometric strength by hand-grip dynamometry; lower body explosive strength by a standing long-jump test; cardiorespiratory fitness by a 20-m shuttle run test.	Serum C3 and C4: Fasted blood samples were analysed by nephelometry.	C3 was negatively correlated with upper body isometric strength, lower body explosive strength and cardiorespiratory fitness. Additionally, C4 was negatively correlated with upper body isometric strength, lower body explosive strength and cardiorespiratory fitness. Regression analysis revealed muscular fitness was negatively associated with C3 and C4 after adjusting for sex, age and pubertal status.

Chen (2021) (21)	30 obese, sedentary women (mean age: 65.4 ± 6.6 years).	Maximal voluntary isometric contraction (MVIC) of the right knee extensors; and performance of functional fitness tests for senior adults (30-s chair stand, 2-min step, 6-m walk, 6-m tandem walk, and balance).	Plasma C1q: blood samples taken at baseline and after the 12-week intervention were drawn into EDTA vacutainers. Samples were centrifuged at 2000 rpm for 10 min and plasma stored at -80°C until further analysis by ELISA kits.	After 12-weeks of descending stair walking training (n=15), the decreased normalised changes in C1q concentrations were associated with MVIC strength, and the 6-m walk fitness tests.
Delgado-Alfonso (2018) (27), UP&DOWN Study	503 children and adolescents (mean age 11.3 ± 3.4-years).	Cardiorespiratory fitness was assessed by a 20-m shuttle run test; muscular fitness was assessed by measuring maximum handgrip strength and the standing long jump; motor ability was assessed by a 4x 10-m shuttle run test.	Serum C3 and C4: fasted blood samples drawn into dried gel and sodium citrate, centrifuged and frozen at −80°C. C3 and C4 was measured by turbidimetry.	In children, 20-m shuttle run, handgrip strength, standing long jump, motor ability and global fitness were predictors of C3 and C4, respectively. In adolescents, only 20-m shuttle run, standing long jump and global fitness were predictors of C4 and no parameters were predictors of C3. C3 and C4 were lower in children with the highest global fitness compared to the least fit tertile; whereas only C3 was lower in adolescents with the highest global fitness compared to the least fit tertile.
Espersen (1996) (33)	8 male, elite swimmers (mean age: 20-years [range: 18 to 22-years]) and 10 age- and sex-matched controls (mean age: 27-years [range: 22 to 40-years]).	A case-control comparison between trained and untrained individuals.	Plasma C3d: blood samples (n = 4) were collected before, immediately after and 2-h, and 24-h after exercise in both athletes and aged-matched controls. Blood was drawn into an EDTA-coated tube and plasma was frozen at −80°C within an hour of collection. Plasma C3d was measured by ELISA.	C3d was not different between elite swimmers and their age- and sex-matched controls.
González-Gil (2018) (42), Healthy Lifestyle in Europe by Nutrition in Adoles- cence (HELENA) Study	659 adolescents (295 boys; 364 girls), of which 127 were overweight or obese and 383 were classified as metabolically healthy.	Physical activity by the International Physical Activity Questionnaire for Adolescents.	Serum C3 and C4: fasted blood samples were drawn and centrifuged at 3500 ×g for 15-mins. Samples were analysed by nephelometry.	There were no statistical analyses conducted that determined the association between physical activity and complement proteins.
González-Gil (2017) (43), Healthy Lifestyle in Europe by Nutrition in Adoles- cence (HELENA) Study	543 adolescents (251 boys; 292 girls).	'Ideal' levels of physical activity were classified as participating in more than 60-mins of self-reported moderate to vigorous exercise per day.	Serum C3 and C4: fasted blood samples were analysed for C3 and C4 by nephelometry.	There were no statistical analyses conducted that determined the association between physical activity and complement proteins.

Karacabey (2005) (57)	40 sportswomen who have played volleyball 3 times per week for at least 5-years and 20 healthy age-matched less active women were enrolled.	A case-control comparison between trained and untrained individuals.	Serum C3 and C4: blood samples were collected once from untrained individuals and five samples were taken in the trained individuals (before exercise and immediately, 4-h, 2-days, and 5-days after exercise). Blood samples were separated as soon as possible and stored at −80°C. C3 and C4 were measured by turbidimetry.	There were no differences in resting C3 or C4 between sportswomen and their healthy age-matched less active controls.
Karacabey (2005) (56)	40 elite male participants who have played volleyball 3 times per week for at least 5-years and 20 healthy-age matched less active male controls.	A case-control comparison between trained and untrained individuals.	Serum C3 and C4: blood samples were collected once from untrained individuals and five samples were taken in the trained individuals (before exercise and immediately, 4-h, 2-days, and 5-days after exercise). Blood handling and analytical procedures were not clearly stated by authors.	There were no differences in resting C3 or C4 between elite men and their healthy age-matched less active controls.
Labayen (2009) (69), The EYHS Study	145 children (74 girls; 71 boys) and 118 adolescents (65 girls; 53 boys) who were 'apparently' healthy, of which 9.9% were overweight or obese.	Cardiorespiratory fitness was determined by an incremental cycling test.	Serum C3 and C4: fasted blood samples were taken from participants in the supine position. Samples were stored at −80°C.	There were no statistical analyses conducted that determined the association between cardiorespiratory fitness and complement proteins.
Lin (2017) (74)	12 older adults who could walk independently or with aids (6 women; 6 men; mean age: 77.6 ± 1.2-years).	Handgrip strength was measured by a handheld dynamometer; cardiorespiratory fitness was assessed by the 6-mins walk test.	Serum C1s subcomponent, C4b-binding protein α chain, C5, C6, C7, C9, factor B and factor H: fasted blood samples were collected into vacutainers containing an anticoagulant, centrifuged at 1500 ×g for 15-mins and stored at −80°C. Serum protein concentrations were measured by mass spectrometry.	There were no statistical analyses conducted that determined the association between cardiorespiratory fitness/handgrip strength and complement proteins.
Martinez-Gomez (2012) (80), The AFINOS Study	183 adolescents (95 boys; 88 girls; mean age: 14.8 ± 1.3-years), of which 24.6% were overweight or obese.	Sedentary time, time spent watching television and physical activity was quantified by 7-days of accelerometery and self-report.	Serum C3 and C4: blood samples were measured by turbidimetry.	C3 and C4 were not associated with sedentary time or television viewing time, when models were adjusted for age, sex, pubertal status, moderate to vigorous physical activity or body mass index.

Martinez-Gomez (2012) (82), Healthy Lifestyle in Europe by Nutrition in Adoles- cence (HELENA) Study	1025 adolescents (476 boys; 549 girls)	Physical activity was measured objectively by accelerometery and subjectively by the International Physical Activity Questionnaire for Adolescents; cardiorespiratory fitness was assessed by the 20-m shuttle test; muscle fitness was measured using a hand-grip strength and a standing long jump; muscular fitness was assessed by a 4x10-m shuttle-run test.	Serum C3 and C4: fasted blood samples were drawn and measured by nephelometry.	Objectively measured vigorous physical activity was negatively associated with C3, independent of body mass index. Furthermore, objectively measured fitness was negatively associated with C3 and C4, independent of body mass index.
Martinez-Gomez (2010) (81), The AFINOS Study	192 adolescents (98 boys; 94 girls; mean age: 14.8 ± 1.3-years), of which 19% were overweight or obese.	Physical activity was quantified by accelerometery; cardiorespiratory fitness was assessed by a 20-m shuttle-run test.	Serum C3 and C4: fasting blood samples were collected, allowed to clot for 1-h, centrifuged and stored at −80°C. C3 and C4 were measured by turbidimetry.	C3 and C4 were not correlated with total, moderate, vigorous, or moderate to vigorous physical activity. However, both C3 and C4 were negatively correlated with cardiorespiratory fitness. C3 and C4 were adjusted for age, sex and pubertal status.
Nieman (1989) (97)	11 male marathon runners, who had completed at least 3 marathons (mean age: 42.7 ± 2.1-years) and 9 less active male age-matched controls (mean age: 44.2 ± 1.2-years).	A case-control comparison between trained and untrained individuals.	Serum C3 and C4: fasted blood samples were collected before, during (every 5-mins) and 5-mins, 10-mins, 15-mins, 30-mins, and 45-mins after exercise, for both trained and untrained. Samples were drawn from a catheter and analysed by nephelometry.	Resting C3 and C4 were lower in athletes compared to non-athletes, respectively. C3 and C4 were not correlated with training load or marathon performance time.
Phillips (2017) (108), The Cork and Kerry Diabetes and Heart Disease Study	396 adults (46% male; mean age: 59.58 ± 5.46-years), of which 317 were overweight or obese.	Physical activity was measured by accelerometery.	Plasma/serum C3: fasted blood samples were collected at baseline and after a 7.5-year follow-up period. Samples were analysed by turbidimetry.	The most sedentary tertial had higher C3 compared to the least sedentary tertile. C3 were lower in the tertiles performing the most light and moderate to vigorous physical activity duration, compared to the tertile that performed the least respective form of physical activity. Once adjusted for age and gender, isotemporal modelling analysis revealed that replacing 30-mins of daily sedentary time with 30-mins of moderate to vigorous physical activity reduced C3.
Puchau (2009) (112)	100 healthy participants (79 women; 21 men; mean age: 20.7 ± 2.7-years), of which 20.4% were overweight.	Physical activity was determined by self-reported time spent practicing sports and whether the participants considered themselves active.	Serum C3: fasted blood samples were immediately centrifuged at 3500 rpm and 4°C for 15-mins and stored at −80°C. C3 was measured by turbidimetry.	Participants with low C3 (<1.085 g/L) were more likely to self-report regular participation in sport (54.9% vs. 30.6%), more time spent practicing sport (3.0 ± 6.5 vs. 1.0 ± 1.9-h per week) and consider themselves active (84.3 vs. 67.3%), compared to participants with high C3 (>1.085 g/L), respectively.

Ruiz (2008) (117), The AVENA Study	416 adolescents, aged 13.0-18.5-years (230 boys; 186 girls; mean age: 15.4 ± 1.4-years), of which 26% were overweight or obese.	Cardiorespiratory fitness was measured by a 20-m shuttle run; muscle strength was measured by a handgrip strength test and the standing broad jump test.	Serum C3 and C4: fasted blood samples were separated and stored at −80°C. C3 and C4 were measured by turbidimetry.	After controlling for sex, age, pubertal status, weight, height, socioeconomic status and cardiorespiratory fitness, C3 was associated with muscle strength; however, no association was evident for C4.
Ruiz (2007) (118), The European Youth Heart Study	142 children (74 boys; 68 girls; mean age: 9.5 ± 0.4-years).	Physical activity was measured by accelerometery; cardiovascular fitness was measured by an incremental cycling test to exhaustion.	Serum C3 and C4: fasted samples were drawn with the participant in a supine position, separated and stored at −80°C. C3 and C4 were measured 'with kits from DakoCytomation'.	After controlling for age, sex and pubertal status, C3 was associated with cardiovascular fitness, but not physical activity. C4 was not associated with physical activity cardiovascular fitness.
Saygin (2006) (123)	15 less active controls (mean age: 22.2 ± 2.7-years), 15 volleyball players (mean age: 20.9 ± 2.2-years) and 15 long distance running athletes (mean age: 21.6 ± 1.9-years).	Cardiorespiratory fitness was estimated by a 20-m shuttle run test; q case-control comparison between trained and untrained individuals.	Serum C3 and C4: blood was measured by turbidimetry.	Long distance running athletes has lower C3, compared to volleyball players and less active controls. Whereas volleyball players had lower C4 compared to less active controls and long-distance runners.
Volp (2012) (142)	157 healthy adults (91 women; 66 men; mean age: 23.3 ± 3.5-years), of which 13.4% were overweight/obese.	A questionnaire on lifestyle habits was used to determine: participation in sports; physical activity patterns; and, a metabolic equivalent index.	Serum C3: fasted blood samples were centrifuged at 2465 ×g at 5°C for 15-mins and stored at −80°C. Serum C3 was measured by turbidimetry.	There were no correlations between C3 and physical activity level.
Watanabe (2015) (145)	131 healthy participants (62 women; 69 men), aged 20 to 81-years.	Muscular strength was measured by knee extensor and flexor dynamometry.	Serum C1q: fasted blood samples were collected at least 48-h after exercise both at the beginning and at the end of the study period. Samples were centrifuged immediately at 1500 ×g at 4°C for 15-mins and stored at −80°C. C1q was measured by ELISA.	Negative correlations were observed between C1q and both isometric peak knee extension power and isometric peak knee flexion power.
Wolach (1998) (149)	Participants were prepubertal elite women gymnasts ($n = 7$) and untrained women ($n = 6$), aged 10 to 12-years.	A case-control comparison between trained and untrained individuals; Participants underwent 20-mins of treadmill running at a heart rate of 170-180 beats per minute.	Serum C1q, C1r, C1s, C2, C3, C4, C5, C6, C7, C8, C9, factor B and properdin: fasted heparinized blood samples were collected before, immediately after, and 24-h after the run in both trained and untrained individuals. Blood was allowed to clot at room temperature for 30-mins, centrifuged at 4°C and stored at −80°C. All complement proteins were measured by RID.	When resting serum was expressed as a ratio of pooled normal sera, resting C1r was elevated in trained compared to untrained girls; whereas C2 (0.81 ± 0.11 vs. 0.98 ± 0.08) and C3 (0.89 ± 0.09 vs. 1.06 ± 0.15) was lower in trained compared to untrained girls. These differences were maintained immediately and 24-h post-exercise. C1q, C1s, C4, C5, C6, C7, C8, C9, factor B nor properdin were not different between groups.
Zhang (2020) (157), China Hainan Centenarian Cohort Study	943 older adults among the longevous population in Hainan, China (81.4% female; mean age: 102.9 ± 2.8-years).	Physical activity was estimated by the Barthel Index of activities of daily living. A case-control comparison between physically dependent and physically independent older adults.	Serum C3 and C4: measured by turbidimetry.	There was no difference in C3 or C4 between the physically dependent and independent groups. When all data was pooled, there was no correlation between C3 or C4 and physical activity. However, C3 was negatively and C4 was positively associated with physical activity in a model that was adjusted for sex, age, body mass index, education, smoking, alcohol consumption, depressive syndromes, and visual and auditory impairments.

studies. C4 was elevated following a training camp in college rugby players (83); whereas, C3 (83, 91, 139, 152) and C4 (91, 139, 152) were unchanged following training intervention that combined more than one type of exercise, compared to baseline. A single (7.1%) study also demonstrated that C3 was lower, and C4 was unchanged, 7-days following the completion of an interval, resistance and judo exercise training intervention (139). Soluble complement proteins in blood at rest collected before and following a military training intervention were the topic of n = 2 (14.3%) studies. C3 was lower following 10-weeks of military training in n = 1 (7.1%) study (66); whereas, opposing findings demonstrated that both C3 and C4 were unchanged following 3-months of military training (53). A single (7.1%) study reported that weight loss during an interval, resistance and judo exercise training intervention did not affect resting C3 or C4 (139).

With training interventions that incorporated only a single type of exercise, n = 2 (14.3%) studies reported soluble complement proteins in blood at rest, following a resistance exercise intervention. C1q was lower following 12-weeks of both resistance exercise (145) or descending stair walking (21); whereas, C1rs-C1-inhibitor complex, C3b, iC3b, C3bBbP, C3c and MAC were unchanged following 4-weeks of resistance exercise (114). A strong negative correlation demonstrated that reductions in C1q were associated with increased muscle cross sectional area following 12-weeks of resistance exercise (145). 21-days of cycling did not alter resting C1-inhibitor, C3 or C4 in professional cyclists; however C4 was elevated after 10-days of the intervention (127). One-month of daily qigong training lowered C3, but not C4, in healthy young students (77). Whereas, 12-weeks of tai chi (3 times per week) elevated C3 precursor and factor H, but reduced C1-inhibitor, C1r subcomponent precursor and factor B, in university judoists (153).

Soluble complement proteins in blood measured at different stages of an athletic season were the topic of n = 2 (14.3%) studies. C3 (24, 92) and C4 (24) were unchanged throughout the athletic season; whereas, a single study reported lower C4 during the season in cross country skiers and speed skaters (92). The latter study also reported lower C3 and C4 one-month following the completion of an athletic season (92).

Habitual physical activity and fitness

The association amongst soluble complement proteins in blood and measurements of habitual physical activity/fitness were the topic of n = 29 (37.7%) studies, and these studies included 9,284 participants (Table 3). The most studied assessments of habitual physical activity/fitness were cardiorespiratory fitness (n = 14; 48.3%) and habitual physical activity levels (n = 13; 46.4%). Assessments of muscular strength (n = 8; 27.6%), a case-control comparison of active and less active controls (n = 7; 25.0%) and assessments of motor ability (n = 2; 7.1%) were also studied. Overweight or obese participants were recruited by n = 12 (41.4%) studies, and a further n = 5 (17.9%) studies exclusively recruited well trained or elite athletes. Whilst all included studies measured habitual physical activity/fitness alongside soluble complement proteins in blood, n = 8 (28.6%) studies did not perform statistical analyses to inform the present review.

Soluble complement proteins in blood between cohorts with different habitual physical activity levels were the topic of n = 3 (10.7%) studies. C3 was lower in participants who engaged in more habitual physical activity, compared to their less active counterparts (108, 112). It was reported that C3 was negatively associated with higher habitual physical activity levels (82); whereas, n = 5 (17.9%) studies reported no correlation between habitual physical activity levels and C3 (80, 81, 118, 142, 157) or C4 (80, 81, 118, 157). A single (3.6%) study employed isotemporal modelling analysis to demonstrate that replacing less active time with 30-mins of moderate to vigorous physical activity would reduce resting C3 in blood at the time of sampling (108).

Soluble complement proteins in blood between cohorts with different fitness and training statuses were the topic of n = 9 (32.1%) studies. C3 and C4 were lower in ‘fit’ (predicted maximal oxygen uptake ≥42 or 35 mL•kg•min-1 for boys and girls, respectively) compared to their ‘unfit’ (predicted maximal oxygen uptake <42 or 35 mL•kg•min-1 for boys and girls, respectively) counterparts (4, 27). Whereas, both C3 and C4 were not different between physically dependent and physically independent older adults (157). C2 (149), C3 (97, 123, 149) and C4 (97, 123) were lower in athletes compared to non-athletic controls; whereas, C1q (149), C1s (149), C3 (56, 57), C3d (33), C4 (56, 57, 149), C5 (149), C6 (149), C7 (149), C8 (149), C9 (149), factor B (149) and properdin (149) were not different between athletes and non-athletic controls. C3 (11, 81, 82, 118) and C4 (11, 81, 82) were negatively associated with cardiorespiratory fitness; whereas, C1q (145), C3 (11, 117) and C4 (11) were negatively associated with muscle strength. Both C3 (3) and C4 (3, 118) were not associated with fitness in n = 2 (7.1%) studies, and a single (3.6%) study reported that C4 was not associated with muscular strength (117). The association between fitness and C4 appears to be weakened by the progression from childhood to adolescence (27).

DISCUSSION

The purpose of this scoping review was to comprehensively explore the relationship between complement proteins in blood and acute bouts of exercise, exercise training, or measurements of habitual physical activity/fitness (Figure 4). n = 77 eligible studies were identified, and these studies included a total of 10,236 participants and 40 complement proteins. Firstly, the C3 and C4 family proteins were the most studied, yet studies investigating proteins that are upstream of these proteins – and therefore exclusive to the classical, lectin or alternative pathways – were less common. Secondly, it appeared that complement was transiently activated immediately following an acute bout of exercise, and complement proteins were also upregulated up to 72-h following resistance exercise and ultra-endurance running, perhaps reflecting muscle-damage induced activation of the classical and alternative complement pathways. On the other hand, exercise training and cardiorespiratory fitness were more commonly associated with a downregulation of C3 family proteins. Lastly, diversity in the sampling procedures, analytical methodologies, exercise intervention and measurements of habitual physical activity/fitness likely contributed to heterogeneity in the findings observed between different studies.

It is well established that an acute bout of strenuous or unaccustomed exercise causes a profound alteration in various plasma proteins (51, 71, 102, 131, 132, 138, 156), yet inconsis-

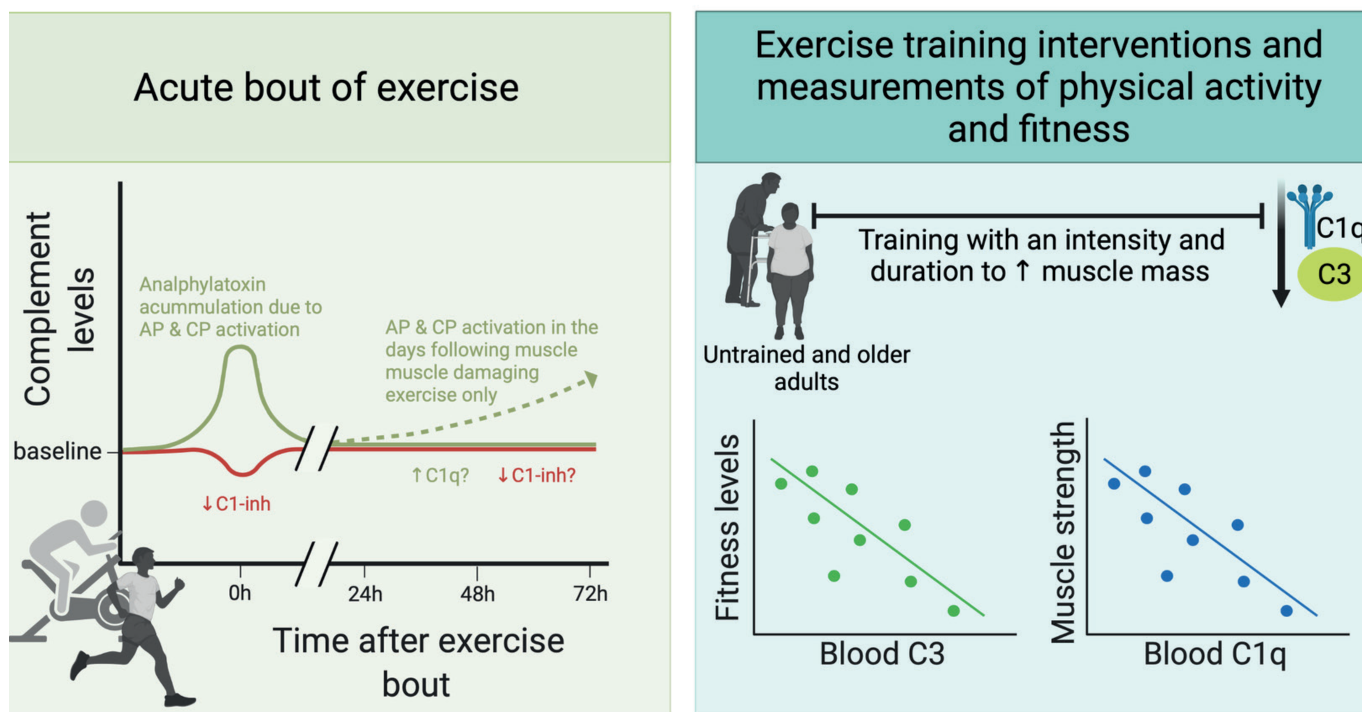

Figure 4. A theoretical graphical summary of the findings of the present systematic scoping review which evaluated the relationship between complement proteins in blood and acute bouts of exercise, exercise training, or measurement of physical activity/fitness among 76 eligible studies, which including 10,206 participants. Firstly (left panel), a consistent finding was that anaphylatoxins (e.g. C3a-des-Arg, C4a-des-Arg and C5a) were increased immediately following an acute bout of exercise in a laboratory setting – potentially due to a transient reduction in the C1-inhibitor (C1-inh), which prevents the formation of both fluid-phase and membrane bound C3 convertases. Whereas ultra-endurance running and resistance exercise – but not exercise which fails to induce muscle damage – increased complement proteins specific to- or downstream- of the classical and alternative pathways (e.g. C1s, factor b, and the C3 and C6 family proteins) for up to 72-h, which may be a result of muscle damage and coincided with a concurrent elevation in the complement activator, CRP. It remains unclear in humans whether other complement proteins specific to the alternative and classical pathways (e.g., C1q) are secreted in excess after muscle-damage, as reported in animal studies (151, 158). Separately (right), C3 in blood was reduced by exercise training and associated with increased fitness, whereas C1q appeared to be negatively associated to muscle strength. This image was created with BioRender.com (Toronto, Canada).

tencies between studies means it remains unclear whether exercise modulates soluble complement proteins in humans (Table 1). One such inconsistency between studies, was the observation that proteins of the classical (C1q and C1-inhibitor), lectin (MBL) and alternative (factor B) pathways were reported to be elevated, lower and unchanged immediately-following an acute bout of exercise (12, 41, 50, 84, 94, 114, 125, 126, 149, 155). More consistent results were reported by studies that employed an acute bout of exercise in a laboratory setting and corrected soluble complement proteins for changes in plasma volume; whereby, the 6 studies which satisified these criterion reported that C3a-des-Arg, C4a-des-Arg and C5a were elevated (16, 18, 30), whereas C3 and C4 were unchanged (32, 92), and C1-inhibitor were lower (50) immediately-post exercise. Reductions in the C1-inhibitor – which prevents the formation of both the membrane-bound (C4b2a) and fluid-phase (C3bBb) C3 convertase (54, 85) and thus is considered a pan-complement inhibitor – could be a mechanism contributing to anaphylatoxin accumulation immediately following an acute bout of exercise in a laboratory setting. Anaphylatoxin accumulation may be further explained by a pH-dependent modulation of the alternative pathway C3 convertase, C3bBb, which is upregulated by lactic acidosis (39, 45, 129). It may alternatively be the case that increased C3a-des-Arg, C4a-des-Arg, and C5a-des-Arg immediately post-exercise reflects muscle-damage induced activation of the classical pathway, albeit at a modest level as many of these laboratory studies did not explicitly seek to induce muscle damage per se (16, 18, 30). Lastly, elevated C4a-des-Arg may arise due to lectin pathway activation (30) – which is in keeping with the observation of increased MBL following ultra-endurance running (12) – though the mechanisms of this process are less clear but could involve changes to gut homeostasis (40). Taken together, it may be that all three pathways can be activated during/immediately after exercise, and further research is needed to elucidate the stimuli for such activation.

While activation of all three complement pathways may occur during or immediately following an acute bout of exercise, release and 'spill over' of C1q from inflammatory/repair responses in muscle (e.g., by macrophages) is likely responsible for the activation of complement – via the classical pathway – for up to 2- to 4-days post-exercise in a muscle damage-dependent manner (151, 158). Indeed, it was consistently demonstrated that an acute bout of cycling or running (duration range: 30-s to 60-mins) did not result in complement secretion or activation – as indicated by unchanged C1-inhibitor, C3, C4, C5a and C6 – 12-h to 6-days following exercise cessation (19, 44, 56, 57, 125). Whereas exercise of a longer duration (e.g. ultra-endurance running) or designed to elicit muscle damage (e.g. resistance training) increased markers of the classical (C1s), as well as C3 and C6 family proteins, up to 72-hours following exercise cessation (12, 101, 126). Notably, no studies included in the present review have investigated the association between complement activation following muscle-damaging exercise and complement activatory proteins, such as CRP (51, 138, 156). However, a single study observed

a similar time-course between increases in the classical complement protein C1s and CRP following a 246-km run (12). Additionally, it was shown that alternative pathway factor B was also increased in the days after ultra-endurance exercise (12, 126). Given that proteins of both the classical and alternative pathways were modulated following ultra-endurance and resistance exercise (12, 101, 126), which coincided with elevated CRP (12), it is plausible that both pathways are involved in skeletal muscle regeneration. This would be in keeping with rodent models which show that regeneration is facilitated by classical complement pathway activation in a factor-B dependent manner (151, 158). Separately, it was observed that a marker of the leptin pathway (MBL) is elevated in the days following ultra-endurance running, and as outlined earlier, the mechanisms underpinning this response are unclear, but could reflect changes to gut homeostasis (40).

In contrast to an acute bout of exercise, exercise training is known to reduce both complement-activating inflammatory proteins (e.g. CRP) and cells capable of secreting complement system proteins (e.g. monocytes) (38, 100). Whilst the majority of studies reported that circulating C3 and C4 were unchanged (24, 53, 77, 83, 91, 92, 127, 139, 152), several studies also reported that C3 or C4 were lower or elevated following exercise training (66, 77, 83, 92, 139). A source of heterogeneity resulted from the duration of exercise intervention. For instance, a 4-week resistance exercise intervention in well-trained young men did not change resting C1 or C3 family proteins in blood (114). Whereas a progressive intervention of resistance training over 12-weeks in older adults lowered C1q, which is a precursor of the classical pathway; an effect that was associated with greater muscle cross-sectional area (145). A reduction of C1q in blood following resistance exercise training is, at first glance, somewhat surprising, as previous research demonstrated that 12-weeks of endurance cycling training increased the intramuscular presence of C1q-secreting M2-like macrophages after an acute bout of resistance exercise (143). However, observations of reduced C1q in blood following resistance exercise could support the notion that C1q is utilised during muscle regeneration – in the presence of the classical complement pathway activator, CRP – through promoting the lysis and removal of damaged myofibers (151, 158), as opposed to being released into circulation. Nonetheless, this emphasises the need for further research to determine whether an acute bout of muscle damaging exercise or a resistance training intervention result in an excessive secretion of C1q by tissue-resident M2-like macrophages, sufficient to increase serum bioavailability – this could have clinical implications, particularly regarding the effectiveness of immunotherapy induced CDC.

A possible further source of heterogeneity amongst included studies that investigated the effects of exercise training on complement system proteins was the fitness of participants at study entry. For instance, complement proteins in blood were unchanged following an exercise intervention in 7/8 (87.5%) studies who recruited participants who were well-trained at baseline (24, 83, 91, 92, 114, 127, 139, 152). Whereas, exercise training interventions modulated soluble complement proteins in 4/4 (100%) studies that recruited untrained or older adult participants (66, 77, 145, 153). As such, exercise training interventions, incorporating sufficient intensity and duration to improve muscle mass, have the potential to modulate complement proteins that are implicated in inflammation or muscle regeneration in untrained or older adult participants.

This notion is supported further by the findings from studies assessing the relationship between complement proteins and measurements of habitual physical activity. The present review found that 5/6 (83.3%) studies found no correlation between habitual physical activity and complement proteins (80, 81, 118, 142, 157); whereas, 3/5 (60%) studies reported that athletes, who were likely to undertake intensive physical activity regularly, were reported to have lower C3 in blood compared to their non-athletic counterparts (97, 123, 149). However, two studies which reported comparable C3 in athletes and controls provided incomplete blood handing/analysis procedures (56, 57). We also found that 5/6 (83.3%) studies reported a negative association between circulating C3 and measures of cardiorespiratory fitness or muscle strength in healthy humans (11, 81, 82, 117, 118). Whereas the majority of studies reported no association between physical activity/fitness and C4 in blood (3, 56, 57, 80, 81, 117, 118, 149, 157). Furthermore, only two studies reported the association between physical activity/fitness and soluble complement proteins other than C3 or C4 (145, 149). As such, future research is required to investigate the association between habitual physical activity, fitness and complement proteins exclusive to specific pathways and/or the MAC.

Areas for further research

Research to date has demonstrated that exercise may enhance anti-cancer immunotherapy through the mobilisation of immune cells (72, 79, 147), modulation of immune checkpoints (13) and vascularisation of the tumour (14). Given that numerous therapeutic monoclonal antibodies used for the treatment of cancers exert cell killing via CDC in a C1q-dependent manner (28, 59), it is promising that an acute bout of exercise may be a means of increasing complement proteins downstream of C1q, which may indicate that exercise can be harnessed to increase the bioavailability of C1q in blood (63). Although complement activation during or immediately following an acute bout of exercise is likely a result of alternative and classical pathway activation (16, 18, 30), complement proteins downstream of C1q are upregulated 12- to 72-hours following resistance and ultra-endurance exercise, perhaps reflecting a dependence on muscle damage and CRP (12, 101, 126), which can be more feasibly achieved in clinical populations using eccentric and/or resistance exercise. These findings are in keeping with rodent models, which have recently demonstrated that C1q is secreted by M2-like macrophages during the resolution of muscle injury, with peak serum C1q occurring 2- to 4-days post-injury (151, 158). However, future research is required to confirm whether the observed complement activation 12- to 72-hours following exercise cessation in healthy humans is the result of increased C1q in blood, which may have clinical implications – for example by augmenting the efficacy of immunotherapy-induced CDC against cancer cells.

The dysregulation of soluble complement proteins is also the feature of numerous chronic health conditions, including diabetes mellitus (5), cardiovascular disease (148), Rheumatoid arthritis (48) and cancer (113). For example, the proliferation of skin and breast cancer cell lines were exacerbated in the presence of pro-inflammatory anaphylatoxins, C3a and C5a, due to upregulated Wnt-β catenin-Sox2 mediated vascu-

lar endothelial growth factor (VEGF) secretion and Akt mediated response gene to complement (RGC)-32 expression, respectively (36, 75). As such, strategies to prevent long-term exposure to anaphylatoxins, such as the C5 targeting monoclonal antibody eculizumab (150), have shown promise and the potential for long-term exercise training to modulate soluble complement proteins is of clinical interest. Whilst studies included in the present review suggested that higher levels of habitual physical activity and cardiorespiratory fitness were associated with reduced C3 in blood (11, 81, 82, 117, 118), no studies determined whether there was an association with fragments of complement (e.g. C3a-des-Arg or C5a-des-Arg), which are indicative of inflammation and complement activation (99). Therefore, future research is warranted to determine whether exercise training elicits an anti-inflammatory effect through the modulation of complement activation, which may have important implications for the prevention and management of chronic diseases associated with complement dysregulation. Nonetheless, it is unknown whether the complement system responds in a similar manner between healthy people and those who have been diagnosed with chronic diseases and/or undergoing treatment that affect complement system proteins (e.g. anti-coagulative therapy; 9). Therefore, future research that investigates the response of soluble complement proteins to exercise training in the context of chronic health conditions is warranted.

Technological advances have also allowed improved biochemical profiling of plasma proteins, which have been successfully used to identify distinct plasma proteomes in states of health and disease in both humans (23, 25, 26, 52, 101) and animal models (90). Global proteomic analyses involve reliable techniques that can produce a large-scale characterisation of the plasma proteome profile whereby protein-protein interactions can be predicted, and therefore serve to provide a broader view of human physiology (47, 107). A proteomics approach could therefore help undercover the mechanisms behind complement changes in response to exercise as this most likely involves several pathways and protein interactions, which are harder to observe in single-targeted protein studies. Recent studies have applied these global proteomic techniques in the context of exercise training and further support the profound alterations that exercise has on the plasma proteome profile (12, 64, 68, 87, 95, 96, 104, 115, 153). Indeed, several studies have demonstrated that the volume of exercise conducted is positively associated with the magnitude of change to the plasma proteome profile and, in turn, the accumulation of complement system proteins (87, 95, 96). While the authors conclude that these changes to complement system proteins are indicative of ‘overreaching’, for reasons highlighted earlier in this review, it may rather reflect ongoing muscle damage/repair and the associated inflammation.

Limitations

Several sources of heterogeneity were identified amongst included studies, which may contribute to the conflicting evidence that currently exists regarding soluble complement proteins in blood and exercise in healthy humans. It was concluded that the primary sources of heterogeneity were discrepancies in blood sampling, handling and analytical procedures. For instance, ethylenediaminetetraacetic acid (EDTA), but not citrate or heparin, is the only anticoagulant to completely inhibit complement activation ex vivo and, therefore, EDTA-plasma is the preferable biological specimen to measure complement concentrations or activation fragments (99, 154). However, 52 of 76 (68.4%) studies measured complement levels and/or activation products in serum. Furthermore, polymer surfaces, including plastics, are established activators of complement ex vivo (46, 128). Despite this, only 1 of 76 (1.3%) studies reportedly used glass laboratory equipment. Lastly, it is well established that exercise expands plasma volume in a modality- and intensity-dependant manner and, therefore, interpreting complement within blood pre- and post-exercise without correction for such expansion could lead to erroneous results (58). For instance, an increased concentration of complement proteins in blood in the presence of an expansion of plasma volume immediately post-exercise could be reported as the complement protein being unchanged from baseline. Nonetheless, only 20 of 76 (26.3%) studies corrected soluble complement proteins for changes in plasma volume pre-, during- or post-exercise (Figure 3). Therefore, it is important that future studies consider these limitations in blood sampling, handling and analytical procedures.

Conclusions

This review demonstrated that C3 and C4 family proteins were the most commonly studied soluble complement proteins in prior research which has investigated the effects of an acute bout of exercise, exercise training or habitual physical activity levels and/or fitness on complement system proteins. There is consistent evidence indicating that long-term exercise training, and the concomitant augmentation of muscle mass and fitness, seems to downregulate C3 family proteins in blood of healthy humans, which is in keeping with the notion that regular exercise exerts anti-inflammatory effects and may have implications for the prevention and treatment of diseases associated with inflammation and complement dysregulation. In addition, it was reported that anaphylatoxins were transiently increased immediately following an acute bout of exercise in healthy humans, likely a result of alternative and classical pathway activation. Whereas soluble complement proteins downstream of the classical complement pathway precursor, C1q, were elevated 12- to 72-hours following resistance and ultra-endurance exercise. Therefore, it may be the case that exercise-induced muscle damage in humans results in elevated C1q secretion from muscle by immune cells. Future studies should explore whether this process can be harnessed to improve the efficacy of cancer immunotherapies that are reliant on CDC as a primary mechanism-of-action.

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Exercise, inflammation and acute cardiovascular events.

Susil Pallikadavath MBChB(hons)[1]; Grace MW Walters MSc[1]; Thomas A Kite MRCP [1]; Matthew PM Graham-Brown PhD[1],; Andrew Ladwiniec MD[1]; Michael Papadakis MD[2]; Gerry P McCann MD[1]; Anvesha Singh PhD[1]

[1] Department of Cardiovascular Sciences, University of Leicester and the NIHR Leicester Biomedical Research Centre, Glenfield Hospital, Leicester, UK.
[2] Cardiovascular Clinical Academic Group, St George's University of London, London, UK.

ABSTRACT

Individuals who participate in regular exercise over time have a markedly reduced risk of cardiovascular disease. Paradoxically, in susceptible individuals with underlying, often undiagnosed, disease states, exercise may acutely increase an individual's risk of cardiovascular events during and immediately following physical exertion. Exercise is thought to evoke conditions that trigger atheromatous plaque rupture or trigger life threatening arrhythmias in individuals with pre-existing, vulnerable coronary artery and inherited cardiovascular disease respectively. This transient increased risk may be driven by the inflammatory trigger provided by physical exertion where exercise is associated with an upregulation of inflammatory mediators in the acute phase. Conversely, habitual exercise can lead to a modulation of the inflammatory response over time. This review explores: exercise related inflammation; acute cardiovascular events related to exercise and strategies to mitigate these risks.

Keywords: Athlete's heart, acute cardiovascular events, exercise immunology.

Corresponding author:
Dr. Susil Pallikadavath
susil.pallikadavath@leicester.ac.uk

INTRODUCTION

The benefits of exercise for cardiovascular health are well recognised. The biggest leap in benefit comes to those that change from no physical activity to modest forms of activity such as brisk walking (1-4). However, the dose-response curves are not fully understood; particularly in those that participate in exercise far above national recommendations (150-300 minutes of moderate-intensity, or 75-150 minutes of vigorous-intensive aerobic activity per week) such as elite athletes (3, 5-7). There is no evidence that healthy individuals participating in exercise are at an increased risk of cardiovascular events. However, in those with susceptible disease states, exercise may provide the inflammatory, haemodynamic and autonomic trigger needed for a cardiac event such as sudden cardiac death (SCD).

This review will explore selected inflammatory responses induced by exercise, their relationship with acute cardiovascular events and strategies that have been outlined by international guidelines in mitigating these events.

ACUTE CARDIOVASCULAR EVENTS

There is a large variation in the estimates of SCD during sport, and it appears that these risks are not homogenous as individuals with susceptible disease states appear to carry a greater burden of risk.

The general population
Overall, the rate of cardiac events in healthy individuals participating in sport is low. In the general population, the incidence of cardiac arrest in 12-45 year olds participating in sport is estimated to be 0.76/100,000 person-years and 0.3/100,000 in those aged 10-90 years (8-10). Based on two large SCD studies assessing out-of-hospital cardiac arrests, the American Heart Association (AHA) report annual incidence rates of 0.5-5.5 and 0.04-0.3 per 100,000 individuals in males and females respectively(10-12). The Registre des Accidents Cardiaques lors des courses d`Endurance reported 17 life-threatening events in 551,880 long distance runners, of which two were fatal (13). While the overall incidence is low, these data suggest that there is heterogeneity in the risk of SCD in exercise which is driven by susceptibility in subpopulations.

There are significant limitations to these data. Capturing sport related SCD is difficult, as it will undercount events in

those performing non-organised, casual sport and elucidating cardiovascular versus non-cardiovascular causes of death can be difficult without autopsy. Furthermore, specific groups are underrepresented in the sports cardiology literature; up to half of published data exclude female participants altogether and non-white individuals have a lower rate of representation when compared to individuals of white ethnicity in other areas of cardiovascular research (14-16).

Susceptible individuals: atherosclerosis versus inherited cardiac disease

Individuals susceptible to acute cardiovascular events during exercise can be generally divided by age. One, young individuals (under 35 years) with inherited cardiac disease such as hypertrophic cardiomyopathy (HCM). Two, older individuals (above 35 years) with underlying atherosclerotic disease. This effect is seen in contemporary data. The FIFA Sudden Death Registry noted that the most common cause of SCD in players above 35 years was coronary disease, whereas in those under 35 years, sudden unexplained death (22%), cardiomyopathies (18%) and other causes (21%) accounted for most deaths (17, 18). Importantly, the incidence of SCD related to exercise appears to be higher in the above 35 years group (3.0 per 100,000 individuals compared to 0.3 per 100,000 in individuals under 35 years) (10, 11). When considering the risks of acute cardiovascular events, these two groups should be discussed separately as the processes of identifying and managing risk may be distinct.

SCD and exercise prescription in inherited disease states

Whilst rare, sudden events in the young tend to receive significant media focus, especially when occurring in apparently healthy individuals in high-profile events. Cardiac arrest and death can be the first presentation of underlying disease such as cardiomyopathy, even in individuals subject to pre-participation screening. Although media focus has centred around the incidence of SCD in organised competition, the risks of acute cardiovascular events are not reserved for those competing at elite level and a higher absolute number of exercise related SCD are observed in non-competitive events (11, 19).

The AHA Council Scientific Statement from 2007 reported HCM and other congenital disease to be the most common cardiac cause of SCD in young athletes (1-51%), usually triggered by ventricular arrhythmias (20, 21). However, more recent data suggests that a large proportion of young athletes with SCD have a structurally normal heart (8, 11). Furthermore, most individuals with HCM do not die during or immediately following exercise. Up to 85% of all HCM related SCD occurs during sedentary activity or rest (22). Similarly, another study found that only 33% of cardiac arrests in individuals with HCM occurred following vigorous physical activity, 43% occurred during daily activities and a further 24% during rest or sleep (23).

Due to the previously perceived risk of SCD associated with exercise, individuals with inherited disease such as HCM have traditionally had conservative exercise recommendations (24). However, the pitfalls of exercise abstinence in HCM are being increasingly recognised and recent studies have investigated the role of exercise prescription in individuals with HCM, as it may improve both cardiorespiratory fitness and arrythmia burden (8, 24). One study enrolled 20 individuals with symptomatic HCM into a supervised exercise programme (25). The investigators showed an increase in functional capacity (4.7 to 7.2 metabolic equivalents) and an improvement in at least one New York Heart Association functional class in 50% of the group, with no concomitant increase in adverse cardiac events (25). As the risk of arrhythmogenesis in HCM is thought to be driven by an acute rise in catecholamines, studies have investigated the characteristics of the adrenergic response. In nine individuals with non-obstructive HCM, a cardiopulmonary exercise study showed that catecholamine levels remain stable during moderate exercise intensity, but increased at higher intensities (26). The authors suggest that cardiopulmonary exercise testing could be a tool used in exercise prescription in individuals with HCM (26). Further trials are underway to evaluate the role of exercise in HCM (ClinicalTrials.gov NCT03335332). Importantly, this may not apply to all types of cardiomyopathy and some studies suggest that in arrhythmogenic right ventricular cardiomyopathy, exercise can accelerate disease progression and trigger arrhythmia (11, 27, 28).

Atherosclerotic disease

In older individuals with underlying atherosclerotic disease, exercise may be associated with a transient rise in the risk of acute myocardial infarction (11, 29). Estimates of the relative risk and odds ratio of myocardial infarction following strenuous physical activity are between 1.1 to 5.7 when compared to very light or no exertion (29-32). In 849 individuals with acute myocardial infarction, 14.1% were triggered by moderate physical activity (33). In adults with coronary artery disease, the wall stress precipitated by tachycardia and transient hypertension can cause extenuation of existing coronary plaque fissures, catecholamine mediated platelet aggregation and more rarely, coronary artery spasm in unhealthy segments, (20). As the population ages, endurance exercise will be taken up by older individuals. Age is an independent driver of atherosclerosis, and the rise in participation is unlikely to be restricted to individuals free of underlying cardiovascular disease. It remains to be seen whether this will be reflected in a rise in acute cardiovascular events during exercise (34).

INFLAMMATORY RESPONSE TO EXERCISE

Acute inflammation as a response to exercise

Exercise is a ‘stressor’ activity. Over time, repeated cycles of damage and repair can improve cardiorespiratory fitness. In the acute setting, the ‘acute phase response’ (APR) to exercise describes the release of pro-inflammatory and anti-inflammatory mediators, including cytokines (35)(Figure 1, Table 1). These rises are transient, and likely determined by the intensity and duration of the exercise, alongside the physiological reserve, including training history of the participant. Siegel et al assessed inflammatory and haemostatic markers in apparently healthy marathon runners who did not take anti-inflammatory medication (36). They compared pre-race measurements against values taken four hours after race completion. Increases were seen in C-reactive protein (CRP), von Willebrand factor, D-dimer and fibrinolytic activity, with a concurrent reduction of fibrinogen (36). In a separate cohort, they demonstrated an increase in white blood cell and platelet counts, with a shortened time to aggregation (36). Von Willebrand factor, D-Dimer and white blood cell count remained elevated the following morning (36). Studies have shown CRP

rise following vigorous physical activity(35). The increase in CRP is thought to be predominantly mediated by interleukins (IL).

IL-6

IL-6 is the initial cytokine released in response to exercise and demonstrates the highest rise but is dependent on the duration, intensity and level of muscle mass recruited in physical exertion and peaks upon completion of the activity (35, 37-39). In vivo, IL-6 is predominantly produced by monocytes, macrophages, fibroblasts, endothelial cells and skeletal muscle, in response to exercise-related muscle injury (39, 40). It was thought that IL-6 is a pro-inflammatory cytokine and is found elevated in states of chronic low grade systemic inflammation, and disease states such as congestive cardiac failure, where it is implicated in myocardial dysfunction and has been shown to predict mortality in females ≥65 years with cardiovascular disease (41-44). However, more contemporary evidence suggests that the rise in IL-6 is due to its anti-inflammatory role in acute exercise. This occurs through its antagonistic relationship with the inflammatory marker, tumor necrosis factor alpha (TNF-α)(37, 43).

TNF-α levels can predispose individuals to endothelial dysfunction, atherosclerosis and extenuate the progression of heart failure(45). TNF-α is found in higher concentrations in diabetes, increasing age, increasing atherosclerosis and predicts mortality(45-47). Whereas insulin sensitivity is improved by IL-6, TNF-α antagonises this effect(37). These moderate increases in IL-6 in response to acute exercise deliver anti-inflammatory effects by opposing TNF-α and stimulating IL-1ra(37, 43). The rise in IL-6 seen in exercise differ to that seen in acute pathological states such as sepsis, as there is no concomitant rise in other markers such as TNF-α and IL-1β(37, 43). However, the rise in IL-6 may not be a homogenous phenomenon to all exercise types. A recent systematic review assessing the change in inflammatory markers in response to moderate and intense exercise demonstrated variable conclusions(48). IL-6 was assessed in 13 of these studies and six studies did not report a significant rise in IL-6 following exercise (48). Stelzer et al and Connolly et al showed a rise in IL-6 in moderately trained amateur athletes and non-competitive men respectively (49, 50). Conversely, there were no changes in IL-6 after exertion in eight endurance athletes(51). These results suggest that the rise in IL-6 may be determined by the type, intensity and duration of exercise.

While IL-6 may have a systemic anti-inflammatory role, it is unclear if this effect confers cardiac protection. IL-6 has been implicated during the inflammatory phase of acute coronary syndromes and associated with haemostasis dysfunction in animal studies (52). García-Salas et al showed that IL-6 predicted adverse events in individuals with non-ST elevation acute coronary syndrome (53). IL-6 is also thought to destabilise atherosclerotic plaques, activate cellular adhesion molecules, have a role in myocardial ischaemia-reperfusion injury and has been investigated as a drug target for reducing inflammation in coronary syndromes (54-57). This rise of IL-6 with exercise appears to correlate with the period of increased susceptibility. Therefore, questions remain whether IL-6 is a potential agent in triggering acute cardiovascular events, particularly in individuals with susceptible disease states. Further studies are needed to investigate causality.

Markers of myocardial injury

Endurance exercise is associated with a transient rise of serum markers traditionally attributed to myocardial injury, including troponin and brain natriuretic peptide (BNP). Meta-analyses have suggested that this may be a common phenomenon (58, 59). Some argue that brief rise and fall of troponin may reflect augmented myocyte permeability that releases free circulating troponins into the extracellular space in response to several mechanisms including inflammation (58, 60). Mousavi et al performed cardiac magnetic resonance (CMR) imaging and biomarker analysis immediately following the completion of a marathon in 14 individuals (61). Not only were myocardial proteins such as troponin, creatine kinase and myoglobin raised, but they demonstrated a reduction in right and left ventricular function. However, there was no evidence of late gadolinium-enhancement on CMR, which led the authors to conclude that a rise in cardiac biomarkers may be attributable to release from the cytosol rather than an indication of myocardial necrosis (61). This view is supported by the characteristics of the rise and fall of troponin following marathon running in other studies, where authors have suggested that biomarker elevation reflects alternative myocardial metabolism (62). Factors that determine the magnitude of this rise are not clear but may be associated with both athlete-related and exercise-related characteristics. For example, Leckie et al assessed the rise in high-sensitivity troponin following a marathon in apparently healthy individuals, individuals with pre-existing heart disease and those who have collapsed at the finish line (63). They found there to be an asymptomatic rise in troponin that correlated with pre-race troponin within the control group (63). Other studies have demonstrated heart rate during exercise to be a predictor of troponin rise following exercise (64). It remains to be seen if the relationship between an individual's training history and current participation may drive the associations that have been observed. More recent data cast doubt on exercise induced troponin rise being a benign event. Aengevaeren et al assessed troponin I in 725 older long-distance walkers (65). They found that a rise in troponin I greater than the 99th percentile predicted mortality and cardiovascular events(65). While clinical and mechanistic conclusions remain speculative at present, it may suggest that troponin elevation is not a benign phenomenon in some groups following exercise.

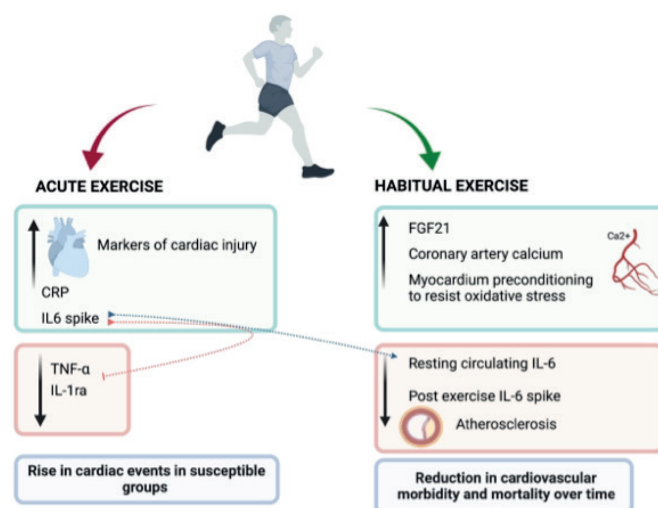

Figure 1. Inflammation related to acute and habitual exercise

Adrenergic surge
Alongside traditional markers of inflammation, the hypothesised mechanism of SCD in diseases such as HCM are thought to be caused by an adrenergic surge. Some argue that there is a higher burden of SCD in sport types with adrenergic surge, such as football and basketball (66, 67). Intense physical activity can trigger the sympathetic nervous system and cause a rapid increase in circulating catecholamines (68). It is thought that this adrenergic surge, increased sympathetic drive, myocardial stretch and microvascular ischaemia can potentiate the risk of arrythmia, and may be a cause of death in individuals with HCM (8, 69). Catecholamines stimulate both α- and β-adrenoreceptors (70). A subsequent rise in intracellular Ca^2+, coronary artery spasm and myocardial ischaemia can increase the risk of malignant arrhythmogenesis (70).

Habitual exercise modulates the inflammatory response
Habitual exercise may lead to regulation of the APR through immunomodulation, upregulation of antioxidative meditators and overall reduction in oxidative stress (71). After prolonged periods of exercise, the distribution profile of inflammatory cytokines, such as IL-1, IL-8 (pro-inflammatory), IL-2, IL-4, IL-10, IL-13 (anti-inflammatory), is balanced towards those with anti-inflammatory properties (71). Periods of exercise may also upregulate the expression of markers associated with favourable cardiac phenotypes. For example, fibroblast growth factor 21 (FGF21) may offer protection against cardiac fibrosis following an MI. In an animal study, exercise training increased FGF21 protein expression and led to improvements in cardiac function by mitigating cardiac fibrosis (72). In mice with ligation of the left anterior descending coronary artery inducing an MI, exercise promoted FGF21 pathways (73).

Over time, physical activity promotes a number of cardioprotective mechanisms including: autonomic sympathetic modulation, a reduction in circulating angiotensin II and upregulation of apelin (74). Animal studies have demonstrated that exercise may mediate cardioprotective effects through a reduction in pro-inflammatory cytokines, reactive oxygen species, superoxides and preconditioning of the cardiac muscle to oppose oxidative and heat stress (75, 76). The role of these inflammatory mediators in cardiovascular inflammation is not fully understood during and following exercise. Whilst interleukins and mediators such as TNF-α may be implicated in the 'response-to-injury' pathway, leading to atherosclerosis progression, further research is needed to explore possible causal links between acute phase inflammatory mediators released in response to exercise and the development of cardiovascular events in athletes (34).

Over time, this modulation in inflammation may protect against disease processes such as atherosclerosis, where exercise can exert anti-inflammatory effects on the endothelium (58, 77). Exercise can upregulate the production of vasodilator molecules and inhibit the expression of oxidative species (58, 77). This view is supported by evidence that habitual exercise is associated with a reduction in the progression of atherosclerosis, and it is widely accepted that exercise promotes coronary artery health. Habitual exercise may modulate the platelet activation seen immediately following exercise, where studies have shown that platelet activation is seen in sedentary subjects but not in physically active subjects (78, 79). Further still, animal studies have shown that exercise may modulate the inflammatory response, such as TNF-α and IL-6 IL-1β, following induced myocardial infarction (80). However, contemporary data suggest that the relationship between exercise and coronary physiology is not fully understood. In 25,485 individuals, when compared to individuals who were inactive, higher levels of physical activity were associated with a more rapid progression of coronary artery calcium (81). This effect was only observed in individuals who had atherosclerosis at baseline, and not seen in individuals who had no detectable coronary artery calcium (81). This finding is consistent with other studies that have demonstrated that athletes had a higher prevalence of atherosclerotic plaques when compared to sedentary individuals; largely comprising of calcified plaques, whereas sedentary individuals harboured plaques of mixed morphology (82). The prognostic implications of these findings remain unclear. On one hand, they could be interpreted to be a pathological consequence of exercise but on the other, they could reflect a more stable plaque that protects against acute coronary syndrome. In the pathogenesis of acute coronary syndromes, it is the rupture of a thin fibrous cap and plaque erosion that account for a large proportion of acute coronary syndromes that cause death (83). Erosion of the calcified nodule and intraplaque haemorrhage only account for a small proportion in comparison (83). It may be that calcified plaques offer biomechanical stability, preventing cap rupture in response to increased wall shear stress or the acute inflammatory response. This view may be supported by molecular imaging studies which have demonstrated that plaque calcification may be a protective response to chronic inflammation, as it stabilises otherwise thin plaques that can predispose vessel occlusion through exposure of the necrotic core and thrombosis (84). Interestingly, the systemic inflammation following a myocardial infarction can itself trigger the onset of further metalloproteinase activity; which can trigger events in non-culprit plaques by catalysing collagen breakdown, while T-cells secrete interferon-y that can inhibit further production of collagen, leaving the fibrous cap of a plaque at risk of rupture (83, 85). This may be why we observe the risks of further infarction in non-culprit lesions to be decreased when revascularisation is performed during the immediate phase of a myocardial infarction (83). This is important in the context of pro-inflammatory events such as exercise, as they may trigger the rupture of prone plaques (11, 86).

Despite many published studies, the limitations in assessing myocardial inflammation should be recognised. Typically, inflammation is measured through serum markers of inflammation and necrosis such as IL-6, TNFα, troponin, high sensitivity C-reactive protein and fibrinogen (43, 87). However, the rise in these markers are not specific to the myocardium (88, 89). Similarly, CMR techniques to assess for oedema, pericardial effusion, hyperaemia (early gadolinium enhancement) and necrosis (late gadolinium enhancement) are not specific to inflammation and the spatial resolution of the technique is relatively limited and so smaller myocardial defects may remain undetected (88, 90). Invasive methods such as an endomyocardial biopsy are not routinely performed and difficult to justify in the context of clinical research, particularly in apparently healthy individuals. Novel methods such as ultrasmall superparamagnetic particles of iron oxide on CMR may help to elucidate inflammatory processes such as macrophage infiltration in the future (88).

Acute cardiovascular events and exercise, Pallikadavath et al.

First author, date	Participants/exercise type	Inflammation assessed	Key, selected results
Acute response to exercise			
Siegel et al (36)	Marathon runners	Biomarkers including: CRP, von Willebrand factor, D-dimer, fibrinogen, fibrinolytic activity, white blood cell. Samples collected morning before, within 4 hours of marathon completion and morning after the race.	- Increase in CRP, von Willebrand factor, D-dimer, fibrinolytic activity and WBC increased within 4 hours of marathon completion when compared to baseline.
Mousavi et al (61)	Marathon runners (n=14)	Biomarkers including: myoglobin, creatine kinase, cardiac troponin T. Samples collected at baseline, immediately after the race, at the time of cardiac imaging and one week following completion. CMR performed at baseline and within three days of marathon completion	-Myoglobin, creatine kinase, and troponin levels were raised following completion of the marathon but normalised after one week. -Right ventricular end-diastolic diameter, end-diastolic area and endo-systolic area increased following completion. - No evidence of delayed enhancement of the left ventricle.
Stelzer et al (49)	Moderately trained amateur athletes, endurance cycling(n=7)	Biomarkers including: IL-6, Fibrinogen, creatine kinase, NT-pro-BNP. Samples collected: 2 days before competition, and within 15 minutes of completion.	-IL-6 and fibrinogen increased following race completion. -NT-pro-BNP, creatine kinase, creatine kinase-MB increased following race completion.
Aengevaeren et al (65)	Walkers (30-55km) (n=725)	Cardiac troponin I. Clinical outcomes include: all-cause mortality and major adverse cardiovascular events. Samples collected: at baseline and within 10 minutes of walking completion.	-Significantly lower survival in individuals with post-exercise troponin I >0.040 ug/L compared with ≤0.040ug/L (HR: 3.21 95%CI=1.79-5.77) in multivariable models. This remained significant when adjusted for baseline troponin.
Response to habitual exercise			
Fischer et al (115)	Healthy untrained males (n=7). 10-week knee extensor endurance training.	IL-6 mRNA expression. Samples collected at rest, end of exercise and two hours after rest.	-Exercise capacity increased. -IL-6 mRNA increased acutely from baseline to post-exercise. However, after the 10-week training period, skeletal IL-6 mRNA expression only increased 8-fold; compared to 76-fold prior to the training period.
Hamer et al (116)	Whitehall II population (n=4289), self-reported physical activity.	Biomarkers including: CRP, IL-6, Baseline markers collected in phase 3 of the study in 1991-93 and compared at phase 7 of the study 2002-04 giving a mean follow-up of 11.3 years.	-Individuals who showed high adherence to physical activity guidelines had lower CRP, IL-6 at follow-up when compared to those who rarely adherence to physical activity guidelines. Individuals who increased their physical activity also showed lower CRP and IL-6 levels when compared to individuals who were stable.

Table 1. Selected studies assessing inflammation in response to acute and habitual exercise.

MITIGATING ACUTE CARDIOVASCULAR EVENTS DURING AND AFTER EXERCISE

Due to the lack of randomised controlled trials and the relative low number of events, there are no data that identify high-risk exercise and subsequent exercise prescription to mitigate acute cardiovascular events, particularly in healthy individuals (20). The issue is further complicated as exercise is not limited to healthy groups or elite athletes. The following section discusses some general recommendations that international guidelines have offered.

Screening

Pre-participation screening is a vast area of discussion and recent updates are outlined in the 2020 European Society of Cardiology (ESC) Guidelines on sports Cardiology(18). Screening programmes aim to identify individuals at risk and offer strategies to reduce this risk, one of which is exclusion from competition. The drive has been to improve the sensitivity and specificity of pre-participation screening programmes in young athletes (18). There is less focus on screening asymptomatic individuals above 35 years, with screening for ischaemia reserved for those with symptoms or those calculated to be at high risk of coronary artery disease by ESC Systematic Coronary Risk Evaluation (SCORE)(18). There are also areas where international guidelines differ. For example, the European guidelines recommend the use of electrocardiograms in the pre-participation screening protocol, whereas the AHA do not (11, 18, 91).

Exercise prescription: gradual progression of training load

Matching workload to training history to activity may be important. Mittleman et al interviewed 1228 individuals with recent myocardial infarction (92). Around 1 in 20 reported heavy exertion in the preceding one hour before myocardial infarction. They also showed that the risk of myocardial infarction during exercise was higher in individuals who were habitually sedentary (92). Other studies also demonstrate that a disproportionate number of cardiovascular events occur in those with a low-training history participating in unfamiliar levels of physical activity (11, 20, 92). International guidelines recommend that training plans should involve incremental increases in training load, as this theoretically improves an individual's conditioning, reducing their risk of acute adverse events (11, 20).

Warm-up/cool down

Warm-up and cool-down routines are recommended, particularly in those with pre-existing cardiac disease (11). Barnard et al assessed 44 asymptomatic males with and without prior warm-up to vigorous exercise (93). Abnormal ECG patterns were observed in 31 individuals when no prior warm-up was performed, 19 of which showed ischaemic changes, whereas no ECG abnormalities were detected when subjects performed incremental increases in exertion levels (93). They separately demonstrated similar findings in 10 healthy men who performed sudden treadmill exercise without warm-up, where three individuals had ST segment depression and a further three had minor ischaemic changes. While experimental data assessing the prognostic value of warm-up routines is unclear, it is thought that sudden intense physical activity could provide conditions for acute cardiovascular events in individuals at-risk.

Avoiding extremes of temperature

In general, the AHA recommend that inactive individuals and those with cardiovascular disease should reduce vigorous physical activity in extremes of temperature (11). Studies report a higher number of cardiovascular events during and after snowfall periods, with up to 7% of admitted acute coronary events in those involved in snow shovelling (94, 95). This may be a consequence of training history and activity mismatch or due to angina caused by coronary vasoconstriction in response to cold temperatures (20). Exercise at hot temperatures is associated with relative tachycardia, due to the thermal load and peripheral vasodilation causing a drop in total peripheral resistance (20). Higher temperatures can precipitate dehydration and electrolyte disturbances, which endurance athletes may be prone to (96).

Recognition of symptoms for professionals and patients

SCD can be the first presentation of underlying cardiovascular disease (97). This is particularly evident in young athletes with cardiomyopathies (97). However, in a prospective cohort of athletic individuals with cardiac arrest between the age of 5-34, up to 29% reported symptoms prior to the arrest and 14% were associated with exertion (98). In a retrospective analysis of 60 sudden deaths among squash players, 45 stated symptoms in the week preceding sudden death, with the most common symptom being chest pain (99). International guidelines recommend that individuals that experience cardiovascular symptoms during exercise should cease physical activity and receive medical review (11, 18). Policy makers may wish to focus on education to participants, coaches, families and school teachers and promote the discussion that cardiovascular symptoms, particularly when associated with exertion, should prompt cessation of activity and medical evaluation.

The role of pharmacological prophylaxis

Studies have suggested that there may be a theoretical role for pharmacological prophylaxis in susceptible individuals (11). Siegel argues that marathon runners may be a subpopulation who are at an increased risk of cardiovascular events during activity periods, and as such may warrant aspirin therapy (100). Siegel points to alterations of platelet granularity and clumping during marathon races as evidence of platelet activation during exercise (100). As aspirin irreversibly inhibits cyclooxygenase-1, subsequently decreasing platelet aggregation through thromboxane A2 modulation, they argue that there may be a primary prevention role in these individuals (101, 102). However, the benefits of aspirin are not homogenous to all population and must be carefully assessed against potential bleeding risks observed in several sub-populations, which is a particular concern in athletes engaged in sport with an increased risk of falls and traumatic injury (103, 104). Currently, the role of prophylactic pharmacotherapy for the prevention of exercise related cardiac events remains speculative and is not recommended (11).

The role of recovery and detraining

The role of recovery and detraining in sports cardiology is generally reserved for diagnosis and treatment of cardiovascular disease. In those with cardiac phenotypes that lie in margins of diagnostic overlap between the athlete's heart and cardiomyopathy (termed the 'grey zone'), detraining may be used to assess regression of ventricular size and wall thickness that may point towards a diagnosis of athlete's heart. In non-clini-

cal practice, recovery is an important aspect of athlete training. Training regimes adopt the functional overreaching principle which sees repeated exercise and subsequent temporary loss in performance followed by a period of rest in the hope that improvements in performance follow. There is little to no data that examines the role of rest in the prevention of acute cardiovascular disease during exercise, beyond the principles of gradual acclimatisation to vigorous activity previously discussed. Clinically, there are areas to explore. The window of immunodepression refers to a transient drop in immunological function in response to exercise, determined by a drop in salivary IgA, natural killer cells and antibody production (105, 106). Periods of repeated exercise may increase the exposure to the window, leading to an increased propensity to develop infections (106). This is compounded when paired with health-related behaviour that increases the risks of infection in athletes such as: travel for competition, training in extreme weather and close human contact (107). Inflammatory processes caused by infections, such as community acquired pneumonia, can increase susceptibility to cardiovascular disease (108). These questions have come into light during the coronavirus disease 2019 (COVID-19) pandemic, as there have been some reports of its association with myocardial injury and myocarditis (109). The general recommendations are that athletes with viral infections should refrain from activity during the acute phase of illness and should be asymptomatic before restarting (110). Even so, there is little data that explores the rates of acute cardiovascular events in the acute illness phase. For athletes, where detraining and commitment to teams and sponsors are important aspects of career progression, further data is important to guide recommendations around rest in acute phases of illness. Beyond acute illness, it is also unclear how rest and recovery can help mitigate the risks of acute cardiovascular events during exercise and how it may modulate exercise related inflammation, particularly in healthy individuals who engage in regular exercise.

Urgent treatment

Although beyond the scope of this review, a key strategy to improve outcomes in the setting of organised sport participation is the ensure the prompt delivery of resuscitation. In the setting of cardiac arrest, this occurs through cardiopulmonary resuscitation and automated external defibrillators (AEDs). In an 18-year observational study, it was reported that neurologically intact survival rates in sports centres with AEDs was 93% compared to 9% in centres without ($p<0.001$)(111). In this study, the availability of an onsite AED was the only independent factor that predicted survival. A recent systematic review showed that in 78% of cases, sports related arrests present with a shockable rhythm (112). Moreover, both bystander cardiopulmonary resuscitation and AED use were associated with survival following exercise related SCD (112). As such, there is a growing need for not only the integration of AEDs in professional and non-professional sport settings, but also improved training in staff and the community (113, 114).

CONCLUSIONS

Exercise evokes an acute inflammatory response that may be a trigger for acute cardiovascular events, but this is rare in those without underlying disease. This is characterised by the release of cytokines and biomarkers associated with cardiac injury. Importantly, the acute inflammatory response appears to be modulated in those that engage in habitual exercise over time, but it remains unclear if this has a causative role in the reduction in cardiovascular risk observed in individuals who engage in frequent exercise. Those with underlying cardiovascular disease such as coronary artery disease or cardiomyopathies have the highest risk of acute events, and these individuals should undergo careful medical assessment prior to exercise recommendations. A gradual and habitual training prescription, early recognition and reporting of cardiac symptoms and adequate recovery are some strategies that may reduce the risk. The data for prevention of acute cardiovascular events triggered by exercise and the role of inflammation remains incomplete and future investigators may wish to explore: exercise types associated with acute cardiovascular events, the role of strategies to mitigate these risks and role of inflammatory markers.

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Effect of high intensity interval training and moderate intensity continuous training on lymphoid, myeloid and inflammatory cells in kidney transplant recipients.

Ganisha M Hutchinson[1], Andrea M Cooper[1] Roseanne E Billany[3], Daniel G D Nixon[2], Nicolette C Bishop[3], Alice C Smith[2]

[1] Department of Respiratory Sciences, University of Leicester, UK
[2] Department of Health Sciences, University of Leicester, UK
[3] School of Sport, Exercise and Health Sciences, Loughborough University, UK

ABSTRACT

Kidney transplantations are seen to be a double-edge sword. Transplantations help to partially restore renal function, however there are a number of health-related co-morbidities associated with transplantation. Cardiovascular disease (CVD), malignancy and infections all limit patient and graft survival. Immunosuppressive medications alter innate and adaptive immunity and can result in immune dysfunction. Over suppression of the immune system can result in infections whereas under suppression can result in graft rejection. Exercise is a known therapeutic intervention with many physiological benefits. Its effects on immune function are not well characterised and may include both positive and negative influences depending on the type, intensity, and duration of the exercise bout. High intensity interval training (HIIT) has become more popular due to it resulting in improvements to traditional and inflammatory markers of cardiovascular (CV) risk in clinical and non-clinical populations. Though these improvements are similar to those seen with moderate intensity exercise, HIIT requires a shorter overall time commitment, whilst improvements can also be seen even with a reduced exercise volume.

The purpose of this study was to explore the physiological and immunological impact of 8-weeks of HIIT and moderate intensity continuous training (MICT) in kidney transplant recipients (KTRs). In addition, the natural variations of immune and inflammatory cells in KTRs and Non-chronic kidney disease (non-CKD) controls over a longitudinal period are explored. Newly developed multi-colour flow cytometry methods were devised to identify and characterise immune cell populations.

Twenty-six KTRs were randomised into one of two HIIT protocols or MICT: HIIT A (n=8; 4-, 2-, and 1-min intervals; 80-90% $\dot{V}O_{2peak}$), HIIT B (n=8, 4×4 min intervals; 80-90% $\dot{V}O_{2peak}$) or MICT (n=8, ~40 min; 50-60% $\dot{V}O_{2peak}$) for 24 supervised sessions on a stationary bike (approx. 3x/week over 8 ± 2 weeks). Blood samples taken pre-training, mid training, post-training and 3 months later. Novel multi-colour flow cytometric panels were developed to characterise lymphoid and myeloid cell population from peripheral blood mononuclear cells. No changes were observed for circulating immune and inflammatory cells over the 8-week interventions.

This feasibility study does not suggest that exercise programmes using HIIT and MICT protocols elicit adverse negative effects on immunity in KTRs. Therefore, such protocols may be immunologically safe for these patients. The inability of the participants to achieve the target exercise intensities may be due to physiological abnormalities in this population which warrants further investigation.

Keywords: High intensity interval training, Kidney transplantation, Flow cytometry, Inflammation, Immune cells

INTRODUCTION

Chronic kidney disease (CKD) is a collective term that arises from heterogeneous disorders that affect the structure and function of the kidney irreversibly, over months or years (1). The most widely used indicator of overall kidney function is glomerular filtration rate (GFR), which associates to the total amount of fluid filtered through the functioning nephrons per unit of time. In clinical settings, equations are used to estimate GFR (eGFR).

Kidney transplantation is the optimal choice of kidney replacement therapy (KRT) in terms of patient survival and quality of life (QoL). Kidney transplantations are classified as a living-donor or cadaveric-donors (deceased-donor). Regardless of the type of donor, blood type testing, tissue typing, cross-matching and serology are conducted to help match a donor to a recipient. In most cases, the patient's existing kidneys are not removed due to increases in surgical morbidity rates. Therefore, donor kidneys are often placed in the iliac fossa. The artery that carries blood to the kidney and the vein that carries blood away is surgically connected to the artery and vein already existing in the pelvis of the recipient. The urethra that carries urine from the kidney is connected to the bladder.

1.1 INCIDENCE AND PREVALENCE

CKD is a global problem and the incidence and prevalence vary greatly by nation. The most recent data puts the total

Corresponding author:
Ganisha M Hutchinson, Ph.D., Crown Bioscience UK, Hillcrest House, Dodgeford Lane, Loughborough, LE12 9TE, United Kingdom, ganisha.hutchinson@crownbio.com

number of KTRs in the UK at 32,624; a prevalence of 501 per million population (pmp) (2). During 2016, a total of 3,328 kidney transplants were performed. The absolute number of living donors had declined in 2016 however, cadaveric donors have increased between 9-13%.

The first year of transplant costs the National Health Service (NHS) £17,000 and £5000 for every subsequent year per patient. During 2016, 2.4% of prevalent transplant patients experienced graft failure and returned to dialysis (2)

The incidence and prevalence of males and females has remained stable for the past ten years. The age at which patients are transplanted has more recently been between 55-59 years old. The incidence and prevalence for KTRs for men and women in the UK was 50.9 and 53.8 years old, respectively (2).

Data suggests there being an increase in renal transplants in non-white ethnic groups in the UK. South Asian group's incidence has increased from 10.5–12.7% from 2010 to 2015 suggesting improved access to transplantation (2).

1.2 CARDIOVASCULAR DISEASE AND CKD

There has been a reduction over time of deaths in transplant patients attributed to CVD (43% in 2003 compared to 22% in 2015;(2). Transplantation has been shown to reduce the risk of CV events compared to those on dialysis (3), however outcomes still remain poorer than in the general populations (4). CVD is an overall term used for disorders of cardiovascular perfusion (e.g. atherosclerosis and ischemic heart disease) and cardiac function (e.g. congestive heart failure and left ventricular hypertrophy). Several CVD risk factors are associated with this patient group, traditional risk factors include co-morbidities whereas non-traditional are suggested to influence morbidity and mortality (table 1).

The American Heart Association considers classifying those with CKD a high-risk group for CVD (5). Although mortality rates have declined for CVD in transplantation to 18% in 2017; due to greater cardiac screening, there is still high prevalence and incidence of CVD. Inflammation has become a strong predictor of CVD and mortality (6-11). KTRs have a high activated inflammatory response due to a decrease in renal clearance of inflammatory cytokines and comorbidities as a result of their CKD resulting in immune dysfunction.

Table 1. Traditional and non-traditional risk factors for cardiovascular disease in KTRsised. This image was created with BioRender.com (Toronto, Canada).

Traditional	Non-traditional
Hypertension	Homocysteine
Dyslipidaemia	Anaemia
Diabetes mellitus	Inflammation
Renal impairment	Proteinuria
Left ventricular hypertrophy	
Lifestyle factors	

1.3 INFLAMMATION

Inflammation is a biological response of the vasculature or tissue to injury, infection, ischemia, and autoimmune diseases. Inflammation occurs through polypeptide cytokines that mediate the inflammatory response through autocrine, paracrine, and endocrine mechanisms. Chemotactic cytokines attract leukocytes and mononuclear cells to sites of injury. These cytokines are classified as either pro-inflammatory or anti-inflammatory and are involved in acute or chronic responses (12). Acute responses are non-specific short term physiological responses to injury, which can result in redness, swelling and pain (13). Chronic inflammation is referred to as slow, long term inflammation lasting months to years leading to tissue damage (9), and is associated with increases in pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-6 and C-reactive protein (CRP) (14-16).

Evidence has shown inflammation to be a mediator in the prevalence of specific inflammatory diseases such as diabetes and metabolic syndrome (10, 17), heart failure and CVD (11), arthritis and joint disease (sarcopenia, osteoporosis), allergies (including infection), and depression and dementia (9, 18). Inflammation in CKD could be a consequence of decreased elimination and increased generation of pro-inflammatory cytokines as a result of decreased GFR. Regardless of the initial cause of inflammation, expression of IL-1, IL-6 (7), IL-10, IL-1 receptor antagonist (IL-1ra) (12) have been reported in experimental studies.

During the acute response, IL-6, TNF-α, interferon-γ (IFN-γ) are elevated, attributed to macrophages and monocytes at inflammatory sites. An anti-inflammatory effect can be seen following IL-6. In gene knockout mice, IL-6 caused an anti-inflammatory response both locally and systemically following endotoxin exposure (19). Whilst in humans, IL-6 infusion resulted in elevations of anti-inflammatory cytokines (IL-10 and IL-1ra) without stimulation of pro-inflammatory cytokines IL-1 and TNF-α, suggesting a protective effect (20). However, when IL-6 concentrations remain chronically high, recruitment of monocytes to the area of inflammation can cause a switch from acute to chronic responses (21). Transplant rejection accompanied with elevated serum IL-6 has been reported within 2 months following transplantation in kidney patients (22), further to this, elevated IL-6 concentrations have been reported in patients with all stages of CKD and RRT (23, 24) likely contributing to CVD (25).

TNF-α is a pro-inflammatory cytokine associated with killing tumour cells and has a role with regulating pro and anti-inflammatory cytokines (26, 27). Evidence suggests upregulation of TNF-α in CKD patients, however the link between TNF-α and CVD mortality is controversial (27). The combination of TNF-α and IL-6 have increased pathogenesis of endothelial dysfunction. A combination of these inflammatory cytokines contributes to mortality and morbidity in KTRs (28).

The role of cytokines is to keep a balance between pro–inflammation and anti-inflammation. IL-10 is one of the most important anti-inflammatory immune-regulating cytokines. IL-10 is produced late in the activation process by T helper 2 cells and can completely prevent antigen-specific T-cell proliferation. The cytokine has a direct stimulatory effect on B-cell activation, proliferation, and differentiation into antibody secreting cells and can inhibit T helper 1 cytokine synthesis. Following pro-inflammatory cytokines, IL-10 reduces the production of chemotactic factors such as IL-8 or CC chemokines that attract further leukocytes to the site of inflammation (29). In KTRs, serum IL-10 was higher in those with impaired transplants than those with stable transplants and healthy controls (30).

1.4 IMMUNE DYSFUNCTION

Despite KTRs suffering from chronic inflammation, these patients are heavily immunosuppressed. The general principle underlying successful immunosuppression in transplantation is to keep a balance between effective prevention and rejection and signs of over-immunosuppression such as infections and malignancies (31). In 2017, it was found that the leading cause of death amongst KTRs were infection (24%) followed by malignancy (22%;(2). Today, the main form of therapy relies of multidrug combinations with synergistic components each with a different mechanism of action.

Rejection of transplants can occur in three phases: hyper acute, acute, and chronic. Hyper acute rejection involves an immediate response from the recipient's immune system against the transplanted tissue and is mainly due to actions against the donor human leukocyte antigen (HLA). Acute rejection is most frequent and is likely to occur 1 to 3 months post-transplant. Foreign antigens from the donor tissue activate the host T cells; cytotoxic T cells infiltrate the organ and begin to destroy the tissue by a release of perforins. Humoral-mediated rejection also occurs as host B-cells produce antibodies against the tissue, which results in vascular damage. Chronic rejection is a slow-developing process that gradually compromises the tissue function. A key feature in this is inflammation of the donor tissue. Activated T cells release cytokines, which recruit monocytes/ macrophages to attack the tissue, by releasing cytotoxic enzymes that inflame the transplanted tissue (32).

1.4.1 T lymphocytes

T lymphocytes originate from the bone marrow that migrate from the thymus for activation, clonal expansion, migration, effector responses and termination. Phenotypically, naïve T cells are small cells that express surface marker such as CD45RA, CCR7, CD62L, CD127 and CD132. These cells migrate within secondary lymphoid organs where they interact with dendritic cells. Effector CD4+ T cells can differentiate into four subcategories: (i) those possessing pro inflammatory effector characteristics (T helper 1 cells), (ii) those possessing regulatory or anti-inflammatory activities (regulatory T cells), (iii) those with a function to promote B cell follicle development (T helper 2 cells) and (iv) those that provide long term memory (central memory T cells).

Previous data has reported lymphopenia occurring and persisting over several years following transplantation (33). Regulatory T cells (Tregs) are classed as CD4+ cells with the expression of IL-2 receptor α chain (CD25). Tregs role of regulating and supressing immune cells via various mechanisms including the production of immunosuppressive cytokines IL-10, transforming factor growth-βeta, IL-34 and IL-35, affecting antigen presenting cell functions of dendritic cells via cell-to-cell contact. Overactivation of Tregs is associated with an increased risk of chronic infections and tumour growth; on the other hand, a deficiency can lead to autoimmunity (34), inflammation and allergy (35).

Memory T cells are defined by surface marker expression of homing receptors CCR7 and L-selectin but can be identified by CD45RO+ CCR7+ and are maintained in peripheral tissues allowing for quick responses to infection. These cells proliferate and produce cytokines in response to stimulation from antigens. It is suggested that KTRs show increases in the number of memory and central memory cells compared to end stage renal disease (ESRD) patients, likely to be attributed to an immune response following transplantation (36, 37). There is little research examining the distribution and function of T cells in KTRs.

1.4.2 Monocytes

Monocytes are a heterogeneous leukocyte type with distinct phenotypic and functional characteristics. Three human monocyte subsets exist based upon CD14 (LPS co-receptor) and CD16 (Fcγ receptor III) expression: classical CD14+CD16− (85%), intermediate CD14+CD16+ (5%) and non-classical CD14-CD16+ (10%) monocytes. Intermediate and non-classical monocytes have been distinguished as pro-inflammatory monocytes due to their capability in producing inflammatory cytokines TNF-α and IL-1β (38, 39). These subsets have been elevated in CVD patients (40) and are regarded as 'pro-atherogenic' due to their inflammatory nature (41).

Previous data has noted a shift in monocyte subsets pre and post transplantation. Increases in non-classical monocytes pre-transplantation has been associated with graft rejection compared to patients who had greater classical monocytes (42, 43). Following 6-months post-transplant a further increase in non-classical monocytes has been reported producing IFN-γ (43). Lee et al (2013) reported that, as CKD advances, the proportion of intermediate monocytes expands, and notably, the expansion of this subset correlated with measures of impaired vascular health (44).

Upon tissue damage or infection, monocytes are rapidly recruited to the affected site, where they can differentiate into macrophages. These macrophages can switch toward a classically activated phenotype (M1; pro-inflammatory phenotype) in response to Th1 cytokines (e.g., IFN-γ) or toward an alternatively activated phenotype (M2; anti-inflammatory phenotype) when exposed to Th2 factors (e.g., IL-4 and IL-13; (45)). Macrophage infiltration has been associated with both acute antibody mediated rejection and acute cellular rejection (46). The study of Grimm et al., (1999) showed that infiltration level of activated macrophages discriminates between clinical and subclinical rejection in kidney allograft patients. Presence of abundant numbers of macrophages are associated with poor graft outcome in kidney and in heart transplantation. Currently, immunosuppressive drugs; i.e. agents that inhibit the anti-donor response, can induce CD163+ M2 macrophages (47).

1.4.3 Immunosuppressive medications

Immunosuppressive medications are vital for keeping a balance between graft rejection and tolerance. These drugs have three effects, the therapeutic effect (suppressing rejection), undesired consequences of immunodeficiency (infection or cancer), and nonimmune toxicity to other tissues. This balance is vulnerable to disruption, leaving KTRs at risk and susceptible to a broad range of bacterial and viral pathogens. Viral infections can cause a 'direct' and 'indirect' effect including immune suppression predisposing patients to other opportunistic infections and oncogenesis (48). There is a wide range of immunosuppressive agents; the main drugs are shown in table 2. Currently, there is no consensus on how to tackle immunosuppressive agents, other than a trial an effect method (49).

According to the Kidney Disease: Improving Global

Outcomes (KDIGO) guidelines, using a combination of Calcineurin inhibitors and Inosine Monophosphate Dehydrogenase Inhibitors is most common. This strategy can decrease the incidence of allograft rejection by 10-20% in the first six months after transplantation (50).

The most frequently given calcineurin inhibitor is tacrolimus, which engages FK506-binding-protein 12 to create a complex that inhibits calcineurin leading to the blockage of nuclear factor of activated T cells (NFAT), which is required for the transcription of genes encoding multiple cytokines including IL-2. Mycophenolic acid is an active component of mycophenolate mofetil (MMF) and blocks the purine synthesis thereby affecting the proliferation of lymphocytes (51). Corticosteroids are also given with the combination of calcineurin and MMF that inhibits the actions of multiple cytokines such as IL-2, TNF-α and IFN-γ. The initial combination of MMF with low dose tacrolimus have shown to have high eGFR (52), reduction in serum cholesterol, LDL-cholesterol and triglycerides (53) in KTRs. However, this is a double-edge sword; the toxic effect of calcineurin inhibitors long-term is a contributor to renal failure. Incidences of diabetes mellitus (54), hyperlipidaemia (55) and anaemia (56) are common in these patients and are also undertreated. Long-term use of MMF with tacrolimus has reduced the incidence of acute rejections (57). However, there has been an increase susceptibility to infections (58), CV events (59) and malignancy, which remain frequent. T cells play a crucial role in transplant tolerance and rejection. A decline in the production of naïve T cells combined with the accumulation of memory and effector T cells, leads to a decreased diversity of the T cell repertoire resulting in an impaired response when challenged (60, 61).

Table 2. Characteristics of small-molecule immunosuppressive drugs. Adapted from Halloran, 2004

Type	Drug	Description	Mechanism
	Azathioprine	Antimetabolite pro-drug, imidazolyl derivative of 6-mercaptopurine	Converted into 6-mercaptopurine in the body to block purine metabolism and DNA synthesis
Calcineurin Inhibitors	Cyclosporine	11 amino acid cyclic peptide	Binds to cyclophilin, inhibits calcineurin phosphatase and T cell activation
	Tacrolimus	Antibiotic from Streptomyces tsukubaensis	Binds to intracellular protein, FKBP-12 to inhibit T-cell activation and IL-12 transcription
Inosine Monophosphate Dehydrogenase Inhibitors	Mycophenolate mofetil	Mycophenolic acid from penicillium molds	Blocks guanosine nucleotide synthesis, inhibits proliferation of T and B cells
Polyclonal Antibodies	Basiliximab	Glycoprotein from mouse fermentation cell line	Binds with CD25, which inhibits IL-2 binding and T cell activation
	Glucocorticoids		Inhibits inflammatory gene transcription

New combinations of immunosuppressant application and protocols are emerging, which can reduce the incidence of rejection, this is often seen as the endpoint in most trials, however the risk of organ function, drug toxicity or immune mechanisms seems to be forgotten. Robust tests for rejection that is T-cell–mediated or antibody-mediated would change clinical management and clinical trials. Measurement of immune responses could guide transplantation management in the same way that measurement of disease activity guides

other fields (e.g. the measurement of lipid levels in the management of hyperlipidemia). This suggests that long-term immunosuppression with clinical surveillance remains best for achieving successful graft and health maintenance.

Immunosuppression and immune over-activation are closely linked with KTRs going from one extreme to the other. Too much immunity can be detrimental causing autoimmunity, whereas insufficient immunity can result in disease susceptibility (16).

1.5 PHYSICAL ACTIVITY

There is strong scientific evidence that regular physical activity reduces the risk of early death, heart disease, stroke, high blood pressure, type 2 diabetes mellitus, breast and colon cancer, whilst also improving anthropometrics and mental health (62, 63). The World Health Organisation (WHO) and the American College of Sport Medicine (ACSM) strongly recommend healthy individuals to partake in physical activity to aim to improve cardiorespiratory and muscular fitness, bone and functional health, and to reduce the risk of diseases, depression and cognitive decline. It is recommended that healthy individuals should partake in at least 150 minutes of moderate intensity exercise and at least 75 minutes of vigorous intensity aerobic physical activity per week. In addition to this, weight bearing activities should be completed twice a week involving all major muscles groups (64).

1.5.1 Physical activity in kidney transplant recipients

Kidney transplantation is burdened by high CV risk because of increased prevalence of traditional and disease-specific CV factors and, consequently, patients are affected by greater morbidity and mortality (5, 65, 66). In KTRs, healthy lifestyle and physical activity are recommended to improve overall morbidity and CV outcomes (67). The International Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines for patients with kidney diseases recommend patients with CKD to undertake aerobic physical activity compatible with CV health and tolerance. It is recommended that at least 30 minutes of moderate intensity physical activity should be undertaken 5 times a week (68). However, given the KTRs are an immunocompromised population, there are no specific exercise guidelines for this population group.

Poor adherence to physical activity and low physical functioning are often a result of reduced muscle mass and/or impaired physical capacity due to co-morbidities or previous effects of dialysis treatment (69). Further specific factors related to kidney transplantation itself, such as renal failure, immunosuppressive treatment leading to myopathy and myalgia resulting in muscle weakness (65), obesity, diabetes, may contribute to the impairment of physical performances in transplanted patients.

1.5.2 Exercise training in CKD and kidney transplant recipients

Transplanted populations are often disregarded from studies and excluded from most physical therapies, hence there is a lack of evidence regarding exercise training and specific guidelines for this population group (70, 71).

1.5.2.1 Exercise type, intensity, and duration

It is well known that physiological adaptations of the CV system and muscle from exercise are dependent on exercise type, intensity and duration (26, 72). Reviews have described these finding in further depth (73-75). The most common form of exercise training used in CKD and heart transplantation research is CV exercise which has increased aerobic capacity and heart rate variability in CKD stages 3-5, haemodialysis and KTRs (76-79). Other exercise training programmes have used a combination of CV and resistance exercise (80-82), as well as solely resistance training (83-85).

Percentage $\dot{V}$O2peak, heart rate peak (HRpeak) or the rating of perceived exertion (RPE)-Borg scale have been used to define exercise intensity for interventions. The majority of studies use a moderate to high intensity protocol between 60-80% HRpeak to induce physiological and muscle adaptations (76, 83, 84, 86, 87). Evidence of this intensity has shown to improve triglyceride/ high density lipoprotein concentrations (88). Regular exercise can be advantageous to reduce symptoms of renal skeletal muscle disorders (89-91). KTRs have an impaired exercise capacity possibly related to immunosuppressive medications inducing muscle atrophy (92, 93). Exercise time on treadmills test, isokinetic muscle power and muscle endurance are reported to increase after 3 months of training, suggesting that skeletal muscle contractile function increases with training in KTRs (92). It is also reported that training counteracts some of the negative effects of glucocorticoid therapy, such as muscle wasting and bodyweight gain, bone metabolism, favouring bone remodelling and may reduce the need for medication to control hypertension (92, 94).

It appears to be clear that exercise regardless of type, intensity or duration has potential benefits to all CKD patients improving health and wellbeing. However, the specific mechanisms behind improvement are not well reported.

Majority of studies report moderate intensity exercise for improvements in CV risk factors and exercise capacity in CKD populations (74, 95-97). Aerobic and resistance based exercise have been shown to be feasible in this population for physical and psychological rehabilitation (98). An evidence based review in solid organ transplant patients assesses moderate to vigorous intensity exercise in patients for improvement in exercise capacity and muscle strength (99), however, there is little to no data for the effects of HIIT on immune and inflammatory markers.

1.5.3 High intensity interval training

High intensity interval training (HIIT) is physical exercise that is characterised by brief, intermittent bursts of vigorous activity, interspersed by periods of rest or low-intensity exercise. Previous models of HIIT used four to six Wingate tests, which involves 30 seconds all-out maximal cycling – separated by four minutes of recovery, totalling only 2 to 3 minutes of very intense exercise per training session (100-103). Reports of improvements in exercise capacity and peripheral adaptations have been reported (104, 105). HIIT has been documented to allow for greater physiological stimulus and adaptations than MICT. These larger benefits seen in the healthy populations are for cardiorespiratory fitness (100, 101, 103), vascular function (106), and skeletal muscle metabolism (101).

More recently, HIIT protocols have been widely used in clinical populations, although protocols may differ to those previously reported. The most widely used HIIT protocol in clinical populations is the 4 x 4 minute at 80-90% HRpeak interspersed with 3-minute periods consisting at 50-60% HRpeak (106-108). This protocol has been deemed safe and tolerable in heart failure and metabolic diseased populations

with improvements in exercise capacity, endothelial function, glucose control and fat metabolism compared to MICT (109, 110). There have been few reports of using HIIT and MICT in CKD populations (111) and haemodialysis patients (97), with improvements in exercise capacity and skeletal muscle synthesis (111). There is superiority in HIIT protocols over MICT in promoting health benefits in diseased populations (107, 112). It has been reported that patients with cardiometabolic disease found an increase in V̇O2peak after HIIT compared to MICT (107), which can be translated as a greater decrease in risk of morbidity and all-cause mortality. Reductions in blood pressure, improved insulin sensitivity, increased nitric oxide availability, improvement in lipid metabolism and increases in PGC-1α have also been reported (107). However, with these studies the sample sizes are often too small, and the length of training is often short. Randomised control trials (RCTs) that compare the superiority of multiple different HIIT protocols in improving aerobic and metabolic parameters is limited (113, 114). Thus, researchers tend to pick a protocol that they can modify the work rest times. More studies are warranted to establish the most efficient protocol for each target subject according to clinical characteristics and fitness level, to improve aerobic capacity and to establish higher adherence. Although the increased application of HIIT in the health and medical fields is expected, its feasibility and safety should be further evaluated in future.

1.5.4 Exercise and inflammation

In the general population, evidence suggests that markers of systemic inflammation are lower in physically active individuals than sedentary individuals (115). Studies have observed an inverse relationship between inflammatory markers and physical activity levels (116).

1.5.4.1 Acute inflammation

A single bout of exercise initiates an inflammatory process in the circulation similar to those induced by infection, sepsis, or trauma (117). There is an increase in the number of circulating leukocytes (mainly neutrophils, lymphocytes, and monocytes) in relation to the intensity and duration of the exercise bout. Increases in inflammatory plasma cytokine concentrations are seen that influence leukocytes such as TNF-α, MCP-1, and IL-1β; and anti-inflammatory cytokines IL-6, IL-10 and IL-1ra. Muscle derived IL-6 increases with exercise and contributes to an increase in circulating levels of IL-6 (118) . Reports in healthy populations have found increases in plasma IL-6 after 30 minutes of running (119) with increases in concentrations up to 100-fold after a marathon (120). It was recently demonstrated that relatively small increases in plasma levels of IL-6 induce the two anti-inflammatory cytokines IL-1ra and IL-10, together with CRP (20) . In CKD populations, moderate intensity exercise has shown to increase concentrations of IL-6 post exercise that have remained elevated up to 1-hour post exercise bout with increases in TNF-α (96, 121). Further data in middle aged participants have found increases in IL-6, IL-8, IL-10 and TNF-α following a single bout of moderate intensity exercise (122).

Humoral or cell-mediated immunity is dependent on the type of cytokines released by T helper cells. Type 1 cells produce IFN-γ and TNF-α which activate macrophages and induce killer mechanisms which include cytotoxic T cells which protect against intracellular pathogens. Whereas type 2 cells produce IL-4, IL-5, IL-10, and IL-13 which promote humoral immunity. Strenuous exercise decreases the percentage of type 1 cells within the circulation, whereas type 2 cells do not change (123). Acute exercise has been shown to decrease the proliferative lymphocyte responses to mitogens (124). With the number of lymphocytes decreasing below pre exercise levels and the CD4+/CD8+ ratio decreasing (125). However, this was not seen in CKD patients after a single bout of walking (121). The T cell repertoire changes following an acute bout of exercise. Both memory and naive T cells increase during exercise, but the mobilisation of memory T cells is greater (125). Many studies have examined the influence of acute exercise on monocytes populations and have typically reported monocytosis following exercise with shifts in monocyte phenotypes to CD14+/CD16+ subsets within the circulation that exhibit pro-inflammatory properties (126, 127) in healthy populations and CKD and clinical populations (128).

1.5.4.2 Chronic inflammation

It is now evident that an acute inflammatory response plays a major role in training adaptations. Regular exercise can lead to lower basal levels of circulating inflammatory markers, as well as reduce the inflammatory response to acute exercise. In CKD populations, 6-months of regular walking resulted in a reduction of basal IL-6, and IL-10 elevations compared to non-exercising controls. This also led to downregulation of lymphocyte and monocyte activation and improvements in systemic inflammation (121).

Adipose tissue can play a role in chronic inflammation through two to three fold increases in plasma concentrations of "adipokines" (TNF-α, IL-6, monocyte chemoattractant protein (MCP) -1, CRP) (12). When under metabolic stress, the secretion of adipocytes results in the recruitment and activation of immune cells. Monocytes and T cells primed by inflammatory molecules TNF-α and IFN-γ lead to monocyte-T cell cross activation (14). The inflammation caused by immune cell cross talk is not a consequence of immune cell infiltration but a consequence of immune cell adipocyte interaction (14). Exercise can have a beneficial effect on adiposity. Non-obese individuals that are inactive have elevated plasma markers of inflammation compared to non-obese active individuals (10, 17, 129), and it appears that the mechanisms through which increases in physical activity has a positive impact upon inflammation are not restricted to changes in adiposity. Observational and interventional studies in the general population and clinical populations have noted lower inflammatory biomarker concentrations in individuals who report performing more frequent and more intense physical activity including lower IL-6 and CRP levels (130-133) . However, interventional study data is less consistent, likely because of lack of control groups, small sample sizes, differences in exercise intensities. Potential mechanisms by which exercise may reduce inflammation may include shifts in monocyte phenotype, especially reductions in immune cell production of inflammatory mediators and immune function adaptations that occur locally in exercised skeletal muscle. Studies have noted in clinical populations, exercise training to reduce intermediate monocyte number as well as TNF-α production (134)

This manuscript is generated from two data sets; The PACE-KD study and the MAP-KD study. The PACE-KD

study's primary aim was to assess the recruitment, retention and adherence to HIIT and MICT programmes in KTRs (manuscript under review). Here we report a secondary analysis aiming to assess the effects of 8-weeks of HIIT and MICT on circulating measures of immunity and inflammation in KTRs. The MAP-KD study was to compare both immune and inflammatory cells in stable KTRs and non-CKD controls over a longitudinal period of 6-months using flow cytometry to assess immune cells and sandwich-based assays to assess cytokine concentrations. A comparison of the two groups may generate comparative values of cell subsets that may be used as a reference tool for graft tolerance and rejection in KTRs.

METHODS

The data included in this report were derived from two clinical studies: PACE-KD (135), a feasibility study of HIIT and MICT training in KTRs, and MAP-KD, a longitudinal observational study assessing circulating immune and inflammatory parameters in KTRs and non-CKD as control data for changes over time. Briefly, in the PACE-KD study KTRs were randomised to 24 thrice weekly supervised HIIT or MICT sessions, matched for workload. Venous blood samples were taken at defined timepoints. In MAP-KD there was no intervention, but venous blood samples were obtained at the same timepoints for comparison. Approval for both studies was granted from the UK National Research Ethics Committee (PACE-KD: 18/EM/0031; MAP-KD: 16/EM/0482). All participants gave written informed consent, and the trial was conducted in accordance with the Declaration of Helsinki. The studies were registered with ISRCTN (PACE-KD: no. 73106458, MAP-KD no. 17122775).

2.1 PARTICIPANTS

All KTRs were recruited from routine kidney transplant outpatient clinics at Leicester General Hospital, United Kingdom. Patients were excluded if they were scheduled surgery or procedures involving anaesthesia, pregnancy, significant disease, or disorder which may put the patient at risk while taking part in the study or may influence the results of the study, and the inability to give informed consent or comply with the testing and training protocol. Eight KTRs were recruited for the MAP-KD study and eight non-CKD individuals known to the research team (i.e., research staff, university and hospital staff or patient family members) were recruited as non-kidney disease controls. Outcome measures were taken at pre-training, mid-training, post-training and 3-months post training for the PACE-KD study, and the same time points for the MAP-KD study: Week 0, Week 4, Week 8 and Week 24.

2.2 EXERCISE TRAINING INTERVENTION

The PACE-KD study exercise intervention has been described in detail previously (135). Briefly, 24 KTRs were randomised to one of two HIIT protocols or MICT: HIIT A (n=8; 4-, 2-, and 1-min intervals; 80-90% $\dot{V}O_{2peak}$), HIIT B (n=8, 4×4 min intervals; 80-90% $\dot{V}O_{2peak}$) or MICT (n=8, ~40 min; 50-60% $\dot{V}O_{2peak}$) for 24 supervised sessions on a stationary bike (approx. 3x/week over 8 ± 2 weeks) and 3 months after the last session.

2.3 BLOOD SAMPLING, HANDLING, AND ANALYSIS

Blood samples were taken on four occasions (pre-training, mid-training, post-training and 3-months post-training).

Peripheral blood mononuclear cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation on Ficoll-Plaque (GE Healthcare, Merck, St Louis, MO USA) (136). The cells were washed twice with sterile PBS. Cells were resuspended in 1mL of complete Dulbecco's Modified Eagle Medium (D-MEM) with 10% heat inactivated fetal bovine serum [500mL D-MEM, 10% fetal bovine serum, 5mL L-glutamine (100mM), 5mL HEPES buffer (1M), 5mL sodium pyruvate (100mM), 10mL MEM amino acids (50X) and 5mL penicillin/ streptomycin (10,000U/ 10mg/mL); Merck, St Louis, MO USA]. One millilitre of freezing media composed of 20% dimethyl sulfoxide (DMSO, Merck, St Louis, MO USA) [40mL heat inactivated fetal bovine serum, 20% DMSO] was then added slowly to the cell suspension. The cells in freezing media were aliquot at 2 x 10^6 cells per mL in 2mL Corning freezing vials (ThermoFisher Scientific, Waltham, MA USA). A CoolCell freezing device (Biocison, Merck, St Louis, MO USA) was used to freeze cell aliquots at -80°C for 24 hours where after cells were stored at -150°C awaiting future analysis (136).

2.3.1 Thawing peripheral blood mononuclear cells

Cell aliquots were thawed at 37°C until a small ice slurry remained. Complete D-MEM was pre-warmed to 37°C, 1mL of pre-warmed media was added to the cell aliquot slowly and the whole was transferred into 15mL conical tube. Cells were washed with 10mL pre-warmed media twice and centrifuged at 400 x g for 10 minutes. The supernatant was decanted, and cells re-suspended in PBS.

2.3.2 Staining peripheral blood mononuclear cells

Firstly, cells were stained with live/dead fixable viability stain (Thermo Fisher Scientific, Waltham, MA USA) by adding the dye to cells suspended in PBS. Cells were incubated for 30 minutes at 4°C protected from light. Cells were then washed in PBS and resuspended in BD staining buffer (BD Bioscience, San Jose, CA USA) and aliquoted into a 96-well U bottomed plate (Greiner CellStar, Merck, St Louis, MO USA). Briefly, BD stain buffer and 2.5µL human Fc blocker (BD Bioscience, San Jose, CA USA) was added to each well and incubated for 10 minutes at room temperature, protected from light. All wells were stained with the relevant antibodies and incubated at 4°C for 30 minutes, protected from light throughout. Cells were washed with flow staining media [500mL Hanks' Balanced Salt Solution, 25mL fetal bovine serum, 5mL L-glutamine (100mM), 5mL HEPES buffer (1M), 5mL sodium pyruvate (100mM), 10mL MEM amino acids (50X) and 5mL penicillin/ streptomycin (10,000U/ 10mg/mL), Merck, St Louis, MO USA] and centrifuged at 200 x g for 5 minutes. Supernatants were discarded; and cells resuspended in media and fixed with formaldehyde solution (Merck, St Louis, MO USA). Cells were transferred into microtubes for flow cytometry analysis.

2.3.3 Flow cytometry collection and analysis

A BD FACSCelesta cytometer with three lasers was used for the study. The instrument configurations were optimised for 12 fluorescence parameters. The blue laser (488nm) stimulated four fluorochromes: PerCP-Cy 5.5 detected via 695/40 bandpass; PE-CF594 (610/20 bandpass); PE (575/25 bandpass) and FITC (530/30 bandpass). The red laser (640nm) stimulated three fluorochromes: APC-CY7 (760/60 bandpass);

APC (670/30 bandpass) and Alexa Fluor 700 (730/45 bandpass). The violet laser (405nm) stimulated five fluorochromes: BV786 (780/60 bandpass); BV650 (670/30 bandpass); BV605 (610/20 bandpass); BV510 (525/50 bandpass) and BV421 (450/40 bandpass). Alternate fluorochromes could be used given the same excitation and band pass filters.

For the 10-colour lymphocyte flow cytometry panel, the following antibodies were used: CD127-PerCP-Cy5.5 (clone SB/199, Biolegend, San Diego, CA USA); CD56-PE (clone CMSSB, Thermo Fisher Scientific, Waltham, MA USA); CD8a-FITC (clone RPA-T8, BD Bioscience, San Jose, CA USA); CD4-APC-Cy7 (clone RPA-T4, Cambridge Bioscience, Cambridge UK); CD3-AF-700 (clone UCHT1, Thermo Fisher Scientific, Waltham, MA USA); CD45RA-APC (clone HI100, Thermo Fisher Scientific, Waltham, MA USA); CD19-BV786 (clone HIB19, BD Bioscience, San Jose, CA USA); CD25-BV650 (clone BC96, Biolegend, San Diego, CA USA); CD45RO-BV605 (clone UCHL1, BD Bioscience, San Jose, CA USA); fixable aqua viability stain- BV510 (Thermo Fisher Scientific, Waltham, MA USA).

For the 4-colour monocyte flow cytometry panel the following antibodies were used: CD14-PerCP-Cy5.5 (clone M5E2, BD Bioscience, San Jose, CA USA); HLA-DR-APC-H7 (clone G46-6, BD Bioscience, San Jose, CA USA); CD16-BV650 (clone 3G8, BD Bioscience, San Jose, CA USA) and fixable aqua viability stain- BV510 (Thermo Fisher Scientific, Waltham, MA USA).

The flow cytometer was calibrated each day prior to sample acquisition using the cytometer setup & tracking kit (CS&T) and rainbow fluorescent 8-peak bead particles (both BD Bioscience, San Jose, CA USA). Data was acquired using instrument installed BD FACSDiva (BD Bioscience, San Jose, CA USA) software and samples were gated based on FSC and SSC parameters followed by live/dead discrimination. The flow cytometry standard (FCS) files were further analysed using FlowJo v10.1 software (FlowJo, LLC). Stained PBMCs which fell within the size FSC and granularity SSC appropriate for leukocytes, were further gated for doublet population and finally cells were gated based on FSC-height and viability before entering full analysis. These steps reduced spurious results and provided confidence in the subsequent analysis.

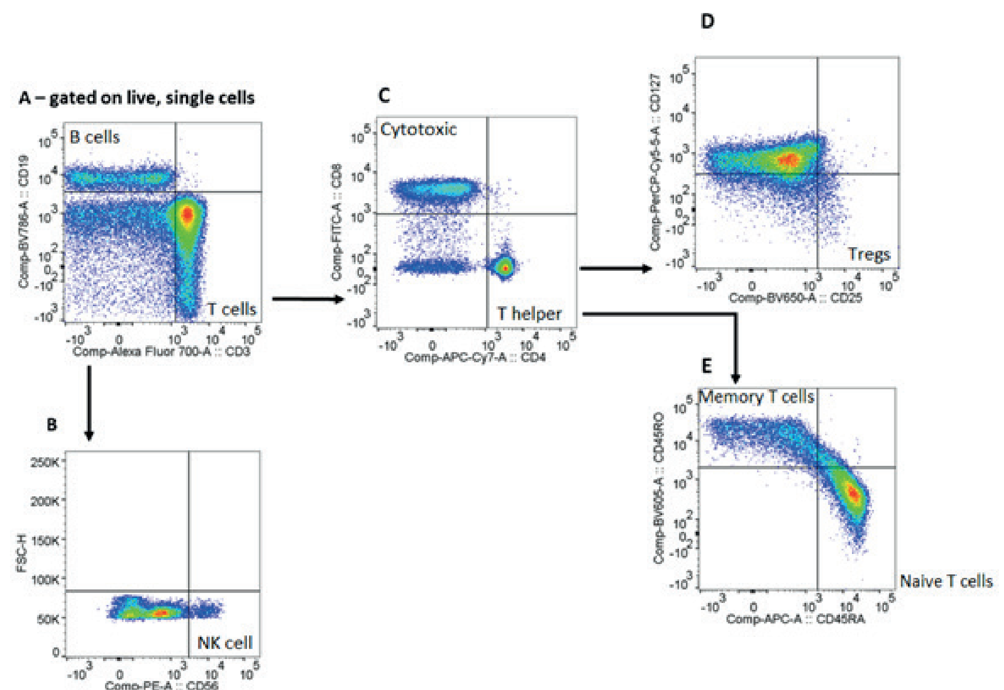


Figure 1. Demonstration of gating strategy used to identify T cell subsets using FlowJo v10.1. Cells are identified as lymphocytes based on size and granularity; single cells are selected based on doublet discrimination. Live, single cells are gated using a fixable viability dye, T cells (CD3+) in the lower right quadrant, B cells (CD3-CD19+) in the upper left quadrant (A), NK cells (CD3-CD56+) in the lower right quadrant (B), gating helper CD4+ T cells in the lower right quadrant and cytotoxic CD8+ in the upper left quadrant (C), initial regulatory T cells (CD25+CD127- (D), naïve (CD45RA) and memory (CD45RO) T cells are gated from CD4+ cell (E).

2.3.4 Lymphocyte analysis

To initially characterise discrete populations within the PBMCs, FSC and SSC properties followed by FSC height and area were used to identify single lymphocytes, from this population BV510 Aqua was used to detect dead cells, all live single cells were gated for further analysis. The single, size and granularity defined, live cells were gated based on CD3 and CD19 expression thereby providing broadly defined B cell (CD19) and T cell (CD3) populations. The single live lymphocyte population that was negative for both CD3 and CD19 was further assessed for expression of CD56 to define a natural killer (NK) population. The single, live lymphocytes expressing CD3 were further analysed for CD4 and CD8 expression to indicate MHC class II and MHC class I restricted T cells, respectively. To address function of the cells CD4+ expressing cells were examined for levels of the receptors for IL-7 (CD127) and IL-2 (CD25) receptors to identify the CD25+/CD127- cells which are thought to represent a regulatory T cell population in humans (Liu et al., 2006). The live single CD3, CD4 expressing T cells were also assessed to determine whether they had been exposed to antigen with CD45RA expression representing naïve T cells and CD45RO representing antigen experienced cells (figure 1; (137).

2.3.5 Monocyte analysis

Cellular differentiation of myeloid populations was achieved using FSC and SSC properties. The viability dye and FSC height and area were used to exclude dead cells and any doublet populations as discussed above. To reduce the complexity of the populations under examination first BV510 conjugated CD3, CD19 and CD56 antibodies were used to place T cells, B cells and NK cells within the same gate as cells staining as non-viable. The remaining cells that expressed both HLA-DR and CD14 were myeloid cells for further analysis. These cells were assessed for their expression of the LPS receptor (CD14) and FcγRIII (CD16) which enable activation, degranulation, phagocytosis, and oxidative burst (138). These populations

are split into three monocyte subsets, non-classical monocytes (CD14-CD16+), intermediate monocytes (CD14+CD16+), the smallest of monocyte sub-population and classical monocytes (CD14+CD16-) which dominate the peripheral circulation (139) (figure 2).

2.3.6 Plasma extraction and analysis

EDTA-treated whole blood was centrifuged at 2500 x g for 10 minutes at 4°C. The plasma obtained was aliquoted into Eppendorf tubes (ThermoFisher Scientific, Waltham, MA USA) and stored at -80°C for later analysis. Plasma samples were analysed by Affinity Biomarker Laboratory (London, United Kingdom) using custom made V plex proinflammatory panel measuring IL-6, IL-10 and TNF-α (Mesoscale Discovery, MD USA), according to the manufacturer's instructions.

3 RESULTS

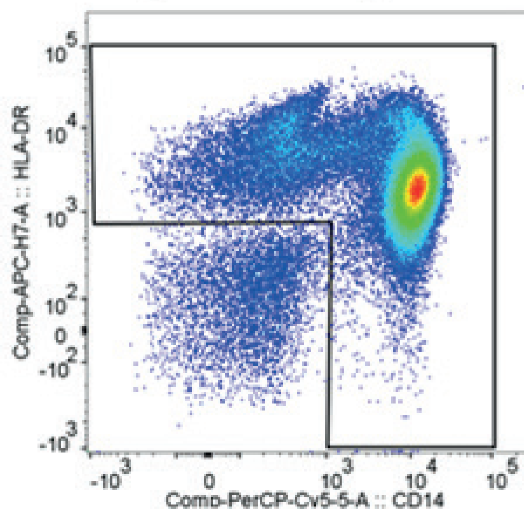
3.1 BASELINE CHARACTERISTICS

Individual characteristics of KTR and non-CKD controls that are included in the comparative analysis (MAP-KD study) can be found in table 3. Patient characteristics of KTRs who took part in the exercise intervention study PACE-KD can be found in table 4.

3.2 THE MAP-KD STUDY

3.2.1 eGFR and leukocyte counts

eGFR was significantly lower in KTRs than non-CKD controls at all timepoints; there was no change over 6-months for eGFR in KTRs or non-CKD controls. KTRs had higher white blood cell (WBC) counts than non-CKD controls at 6-months. Neutrophil counts were greater in KTRs than non-CKD controls at week 4 and 6-months. No differences were found between or within groups for lymphocyte or monocyte counts (P>.05; table 5).



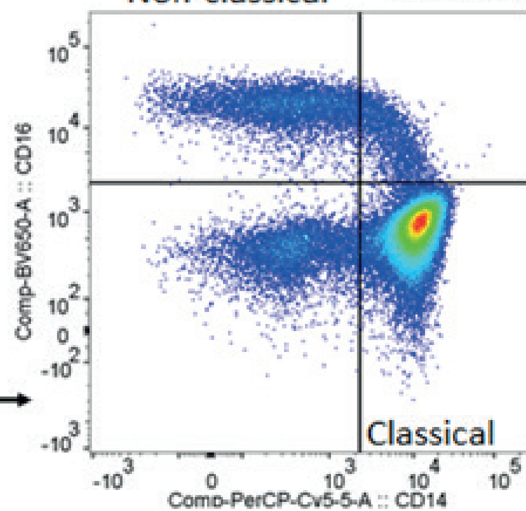


Figure 2. Demonstration of gating strategy used to identify myeloid cells using FlowJo V10.1. Cells are identified to be monocytes based on size and granularity; single cells gated for doublet discrimination, live cells are gated for analysis, CD3+, CD19+ and CD56+ cells are excluded from the analysis. HLA-DR+CD14- cells are gated to remove any other population (A). Monocyte subsets: upper left quadrant non-classical (CD14-CD16+), upper right quadrant intermediate (CD14+CD16+), lower right quadrant classical (CD14+CD16-; B).

2.3.7 Statistical analysis

Data are presented as median (Mdn) and interquartile range (IQR) unless stated otherwise. Participant characteristics were examined using a one-way analysis of variance (ANOVA) to assess differences between the groups at pre-training. Data was inspected for normality using the Shapiro-Wilk test. For non-normally distributed data a non-parametric equivalent was used as appropriate. Effect sizes (ES) are provided on all significant data to give an indication of the proportion of the variance attributed to groups using Rosenthal's definition r = 0.10, 0.30, 0.50 representing 'small', 'medium' and 'large' effects, respectively (140). All statistical analysis was performed on Statistical Package for Social Sciences (SPSS v.25, IBM, NY, USA). Graphs were drawn using GraphPad Prism (v7. GraphPad Software Inc, CA, USA). Statistical significance was accepted at the P < .05 level.

3.2.2 Markers of cellular inflammation

The absolute number of cells and the proportion of cells are given for each marker.

Lymphocyte subsets (figure 3)

T lymphocytes

Non-CKD controls had higher absolute CD3+ cell counts than KTRs at 6-months (P=.040, ES=-0.53). No change for CD3+ cells were seen for KTRs and non-CKD controls over 6-months.

There were no differences observed between KTRs and non-CKD controls for the proportion of CD3+ lymphocytes at week 0, week 4, week 8 and 6-months. However, the proportion of CD3+ cells decreased at 6-months compared to week 0 for non-CKD controls (P=.012, ES=-0.95).

Table 3. Participant characteristics for MAP-KD (n = 16)

	KTRs (n=8)	Non-CKD (n=8)	*P* value
Sex (males)	5 (63)	2 (25)	.149
Age (years)	54 ± 12	42 ± 19	.139
Weight (kg)	78 ± 24	70 ± 11	.407
Height (cm)	173 ± 15	170 ± 11	.609
BMI (kg·m^2)	26 ± 5	24 ± 4	.576
eGFR (ml·min·1.73m^2)	60 ± 12	>90	<.0001*
Time since transplant (months)	84 ± 60	n/a	n/a
Medications			
CNI	8 (100)	n/a	n/a
Steroid	2 (25)	n/a	n/a
Antihypertensive	7 (88)	n/a	n/a
Diabetes	3 (38)	n/a	n/a
Statins	8 (100)	n/a	n/a

Abbreviations: BMI; body mass index, eGFR; estimated glomerular filtration rate, CNI; calcineurin inhibitor
Data are presented as mean ± SD
Notes: categorical values are expressed as integer (% of n)
*denotes significant difference KTRs eGFR < non-CKD controls eGFR (P < .05)

B lymphocytes
No differences were observed between or within groups for the absolute number or proportion of CD19+ lymphocytes.

NK cells
The absolute number and proportion of CD56+ cells for KTRs decreased week 4 (P=.016, ES=-0.84) and at 6-months (P=.016, ES=-0.89). There were decreases for absolute CD56+ cells for non-CKD controls at week 8 (P=.008, ES=-0.89) and 6-months (P=.008, ES=-0.89), but not for the proportion of cells.

T helper cells, Cytotoxic T cells and Regulatory T cells (figure 4)
Non-CKD controls had greater number of CD4+ helper cells then KTRs at 6-months (P=.006, ES=-0.68). There were no significant differences over 6-months for either group. The proportion of CD4+ helper cells were greater in non-CKD controls than KTRs at week 4 (P=.040, ES=-0.29) and 6-months (P=.040, ES=-0.53). There were no changes over 6-months for KTRs and non-CKD controls.

No differences for the absolute number of CD8+ cytotoxic cells were observed between or within groups. The proportion of CD8+ cytotoxic cells were greater in KTRs than non-CKD controls at 6-months (P=.021, ES=-0.59). CD8+ cytotoxic cell frequency decreased at 6-months for non-CKD controls (P=.012, ES=-0.95).

There were no differences for the absolute number or proportion of regulatory T cells between the groups at week 0, week 4, week 8 or 6-months. Regulatory T cells decreased at 6-months for KTRs (P=.016, ES=-0.89) and decreased for non-CKD controls at week 8 (P=.008, ES= 0.89) and 6-months (P=.008, ES=-0.89). The proportion of regulatory T cells decreased at week 8 for KTRs (P=.0.12, ES=-0.89) and non-CKD controls (P=.0.12, ES=-0.89) and at 6-months for non-CKD controls (P=.012, ES=-0.89).

Naïve and Memory T cells (figure 5)
No differences were observed for absolute number or proportion of naïve T cells between or within groups. No differences were observed between groups for the absolute number or proportion of memory T cells. The absolute and proportion of CD45RO+ cells decreased at 6-months for KTRs (P=.016, ES=-0.89).

Monocyte subsets (figure 6)
There were no differences in the absolute number or proportion of non-classical and classical monocyte subsets within or between groups. The absolute number of intermediate monocytes decreased for KTRs at 4 weeks (P=.008, ES=-0.89), but no changes were observed for non-CKD controls.

3.2.3 Markers of circulating inflammation (table 6)
IL-6 concentrations were greater in KTRs than in non-CKD controls at week 0 and week 4. There was no difference for

Table 4. Participant characteristics for PACE-KD

Variable	All (N=24)	HIIT A (n=8)	HIIT B (n=8)	MICT (n=8)
Sex (male)	16 (67)	3 (38)	6 (75)	7 (88)
Ethnicity				
White	18 (75)	5 (63)	5 (63)	8 (100)
Indian	3 (13)	2 (25)	1 (13)	0 (0)
White-Black Caribbean	1 (4)	0 (0)	1 (13)	0 (0)
African	1 (4)	0 (0)	1 (13)	0 (0)
Pakistani	1 (4)	1 (13)	0 (0)	0 (0)
Body mass (kg)	79.7 ± 19.7	68.5 ± 15.6	84.1 ± 24	86.5 ± 15.5
BMI	27.2 ± 5.6	25.9 ± 5.4	28.5 ± 7.1	27.1 ± 4.3
$\dot{V}O_{2\ peak}$ (L/min)	1.99 ± 0.70	1.56 ± 0.30	2.09 ± 0.86	2.33 ± 0.65
$\dot{V}O_{2\ peak}$ (mL/kg^{-1}/min^{-1})	25.28 ± 7.48	23.69 ± 6.29	24.65 ± 7.67	27.5 ± 8.73
Systolic blood pressure (mmHg)	133 ± 14	127 ± 11	135 ± 17	137 ± 13
Diastolic blood pressure (mmHg)	85 ± 9	86 ± 6	81 ± 6	88 ± 11
eGFR (mL/min/1.73 m^2)	58 ± 19	62 ± 18	57 ± 22	55 ± 19
Serum creatinine (mmol/L)	125 ± 49	104 ± 27	133 ± 60	138 ± 53
Kidney transplant vintage (months)†	35 ± 52	12 ± 20	44 ± 86	44 ± 35
Medication				
CNI	24 (100)	8 (100)	8 (100)	8 (100)
Steroid	11 (46)	6 (75)	2 (25)	3 (38)
Anti-hypertensive	22 (92)	7 (88)	7 (88)	8 (100)
Anti-Diabetes	5 (21)	1 (13)	3 (38)	1 (13)
Statins	15 (63)	4 (50)	5 (63)	6 (75)

Abbreviations: CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; HIIT, high intensity interval training; MICT, moderate intensity continuous training
Notes: Unless otherwise indicated, values for categorical variables are expressed as integer (% of n); values for continuous variables as mean ± SD.
†median (IQ), data is non-significant between groups

IL-6 concentrations in KTRs or non-CKD control over 6 months. There were no differences between or within groups for IL-10 concentrations. TNF-α concentrations were greater in KTRs than in non-CKD controls at week 0, week 4, week 8 and at 6-months. There was no difference for TNF-α concentration over time for KTRs or non-CKD controls.

3.3 THE PACE-KD STUDY

3.3.1 Leukocyte counts

Total leukocyte counts did not differ between groups pre-training or post training. In the HIIT A group, total WBC significantly increased from pre-training to post-training. Conversely, total leukocyte counts in HIIT B and MICT did not change from pre to post-training. There were no changes between groups for neutrophil, lymphocyte, or monocyte counts (P>.05).

Table 5. Summary of eGFR data (mL.min.1.73m^2) and leukocyte data ($x10^9$/L) at Week 0, Week 4, Week 8 and 6-months for KTRs and healthy controls

Table 5. Summary of eGFR data (mL.min.1.73m^2) and leukocyte data ($x10^9$/L) at Week 0, Week 4, Week 8 and 6-months for KTRs and healthy controls

	KTRs Mdn [IQR]	Non-CKD Mdn [IQR]	ES	P value
eGFR				
Week 0	59 [49 - 72]	> 90	-0.86	< .001*
Week 4	65 [52 - 70]	> 90	-0.80	< .001*
Week 8	67 [55 - 86]	> 90	-0.80	< .001*
6-months	61 [52 - 70]	>90	-0.69	< .001*
Total WBC				
Week 0	6.3 [5 - 8]	5.1 [4 - 6]	-0.34	.172
Week 4	7.9 [6 - 9]	5.0 [4 - 7]	-0.36	.084
Week 8	6.2 [5 - 8]	5.2 [4 - 6]	-0.39	.112
6-months	7.4 [6 - 8]	5.0 [4 - 6]	-0.64	.025*
Neutrophils				
Week 0	3.7 [2.7 - 4.5]	2.5 [1.9 - 3.5]	-0.36	.154
Week 4	4.8 [3.3 - 5.1]	2.8 [2.1 - 4.2]	-0.56	.038*
Week 8	3.8 [2.2 - 5.1]	2.7 [2.2 - 3.5]	-0.26	.315
6-months	4.4 [3.1 - 5.4]	2.5 [2.2 - 3.5]	-0.69	.015*
Lymphocytes				
Week 0	2.1 [1.5 - 2.5]	1.8 [1.6 - 2.1]	-0.18	.505
Week 4	1.9 [1.5 - 2.3]	1.5 [1.6 - 2.0]	-0.15	.620
Week 8	2.2 [1.3 - 2.3]	1.8 [1.4 - 2.0]	-0.23	.367
6-months	1.7 [1.3 - 2.0]	1.6 [1.4 - 1.8]	-0.18	.589
Monocytes				
Week 0	0.4 [0.3 - 0.5]	0.3 [0.1 - 0.4]	-0.29	.262
Week 4	0.4 [0.4 - 0.5]	0.3 [0.3 - 0.4]	-0.51	.059
Week 8	0.3 [0.3 - 0.5]	0.3 [0.3 - 0.4]	-0.26	.314
6-months	0.4 [0.3 - 0.6]	0.3 [0.3 - 0.4]	-0.41	.177

KTR; Kidney transplant recipients (n = 8), non-CKD control (n = 8), non-normally distributed data presented as median and interquartile range; Mann-Whitney U test between KTRs and healthy controls, ES; effect sizes, $P < .05$
*denotes significant difference between groups

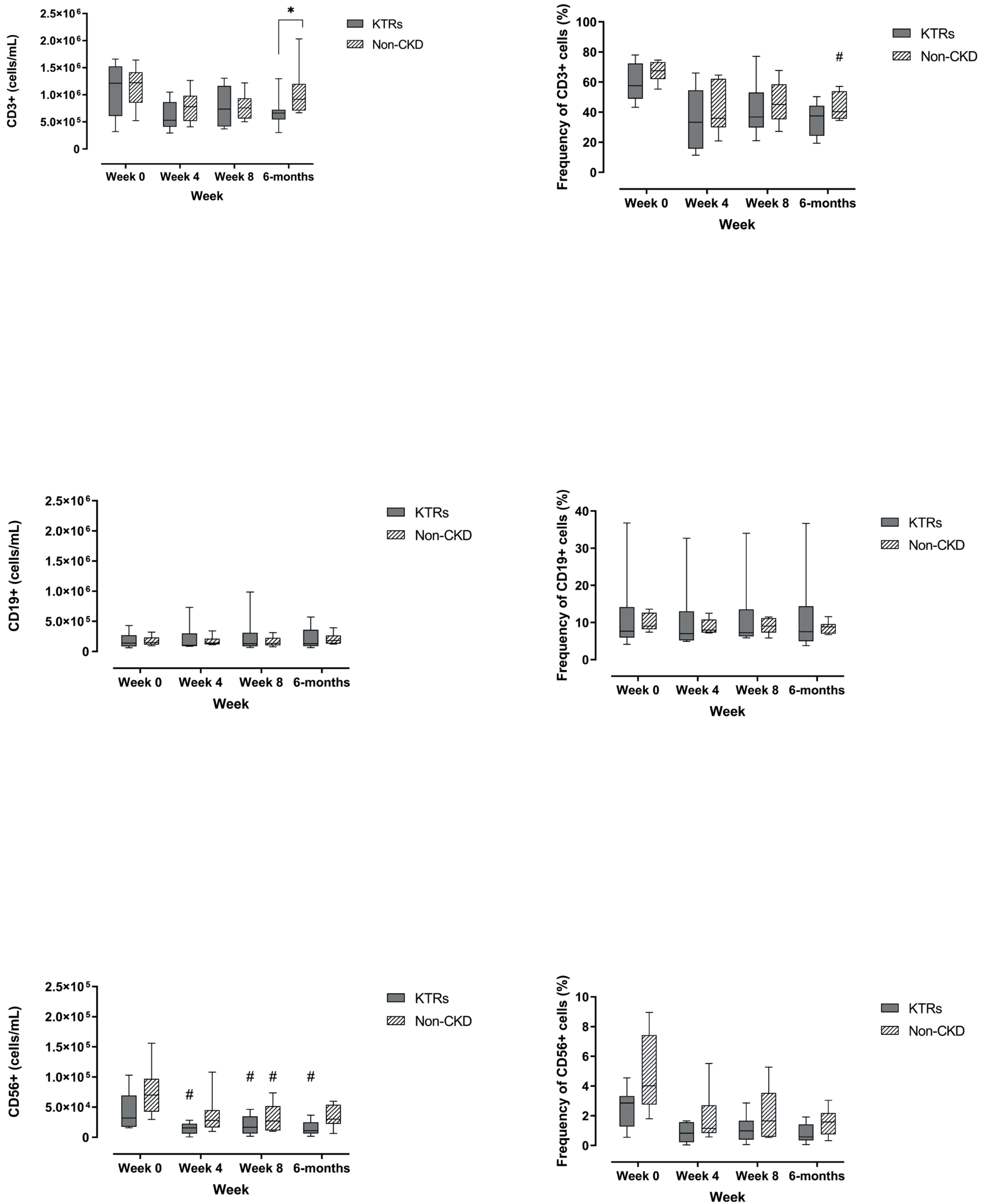


Figure 3. Cell numbers (cells/mL) and cell proportion (%) for CD3+, CD19+, CD56+ cells at week 0, week 4, week 8 and 6-months for KTRs and Non-CKD controls KTR; kidney transplant recipients, non-CKD; non-chronic kidney disease, non-normally distributed data presented as median and interquartile range. # Denotes significant difference from week 0 for Non-CKD
* Denotes a significant difference between groups, non-CKD greater KTRs

Table 6. Summary of cytokine data (ng/L) at Week 0, Week 4, Week 8 and 6-months for KTRs and non-CKD controls

	KTRs (n=8)	Non-CKD (n=8)	*ES*	*P value*
IL-6				
Week 0	0.86 [0.64-1.38]	0.52 [0.37-0.64]	0.57	.021*
Week 4	0.86 [0.73-1.50]	0.40 [0.35-0.51]	0.66	.011*
Week 8	0.97 [0.48-1.66]	0.50 [0.33-0.70]	0.36	.161
6-months	0.90 [0.71-2.35]	0.50 [0.36-0.83]	0.51	.059
IL-10				
Week 0	0.51 [0.37-0.61]	0.44 [0.32-0.86]	0.13	.645
Week 4	0.51 [0.32-0.62]	0.36 [0.34-0.60]	0.11	.710
Week 8	0.55 [0.36-0.64]	0.41 [0.32-0.52]	0.31	.234
6-months	0.54 [0.42-0.64]	0.43 [0.36-0.74]	0.17	.549
TNF- α				
Week 0	3.82 [3.13-4.14]	2.49 [2.29-2.99]	0.70	.003*
Week 4	3.62 [3.32-3.99]	2.47 [2.01-2.74]	0.83	.001*
Week 8	3.79 [2.90-4.02]	2.50 [2.09-2.79]	0.73	.002*
6-months	3.76 [3.02-4.31]	2.62 [2.48-2.93]	0.58	.029*

Data presented as median and interquartile range for all groups * denotes significant difference between KTRs and non-CKD (P<.05)

3.3.2 Markers of cellular inflammation
Lymphocyte subsets (figure 7-10)
There were no differences for absolute number or proportion of CD3+, CD19+, CD56+, CD4+, CD8+ and CD45RO+ between or within groups over time. HIIT B had greater naive T cells than MICT at 3-months post training (P=.014, ES=-0.81). Regulatory T cells were greater in MICT than HIIT A at pre-training (P=.009, ES=-0.69).

Monocyte subsets
There were no differences for absolute number or proportion of monocyte subsets between or within groups over time (P>.05).

3.3.3 Markers of circulating inflammation
No differences were observed for IL-6, IL-10 or TNF-α between or within groups over time (P>.05).

4 DISCUSSION

The main aims of this manuscript was to discuss and then explore the interaction of immune and inflammatory cells in relation to exercise in KTRs. This manuscript comprised of two studies: MAP-KD; in which the main aim was to observe immune and inflammatory cells in stable KTRs and non-CKD controls over a longitudinal period of 6 months to provide comparative data for the PACE-KD trial; an 8 week exercise intervention study exploring HIIT and MICT in KTRs.

In MAP-KD, the main findings were that eGFR and T cell subsets were all higher in non-CKD controls than KTRs. TNF-α and IL-6 were greater in KTRs then non-CKD controls. Following 8-weeks of HIIT and MICT, there were no differences in immune and inflammatory cells in KTRs.

4.1 INFLAMMATION
In the present study, there was an increase in the percentage of CD4+ cells in healthy controls and an increased trend in CD8+ cells in KTRs, but a lack of difference in both groups over time. Previous literature has found no differences between healthy controls and KTRs in the percentage or absolute number of CD4+ and CD8+ T cells two and five years post transplantation (141). No difference between these populations could be a result of optimum immunosuppressive medication regimes. Immunosuppressive medication dosage is initially calculated on a mg/kg bodyweight basis, after a trial-and-error approach is accepted (49), the KTRs in the present study were transplanted 7 ± 5 years ago, and therefore it may be fair to suggest that that optimal dosages of immunosuppressants for this group have been obtained, it may be plausible to suggest that lack of variation over time in immune cell markers for KTRs may also be a result of optimised immunosuppressive medications.

Monocyte subsets percentages were not different between KTRs and non-CKD controls. Patients with ESRD have been shown to have high proportions of circulating non-classical monocytes (142). However, in patients 6-months post renal transplant, there have been observations of increases percent-

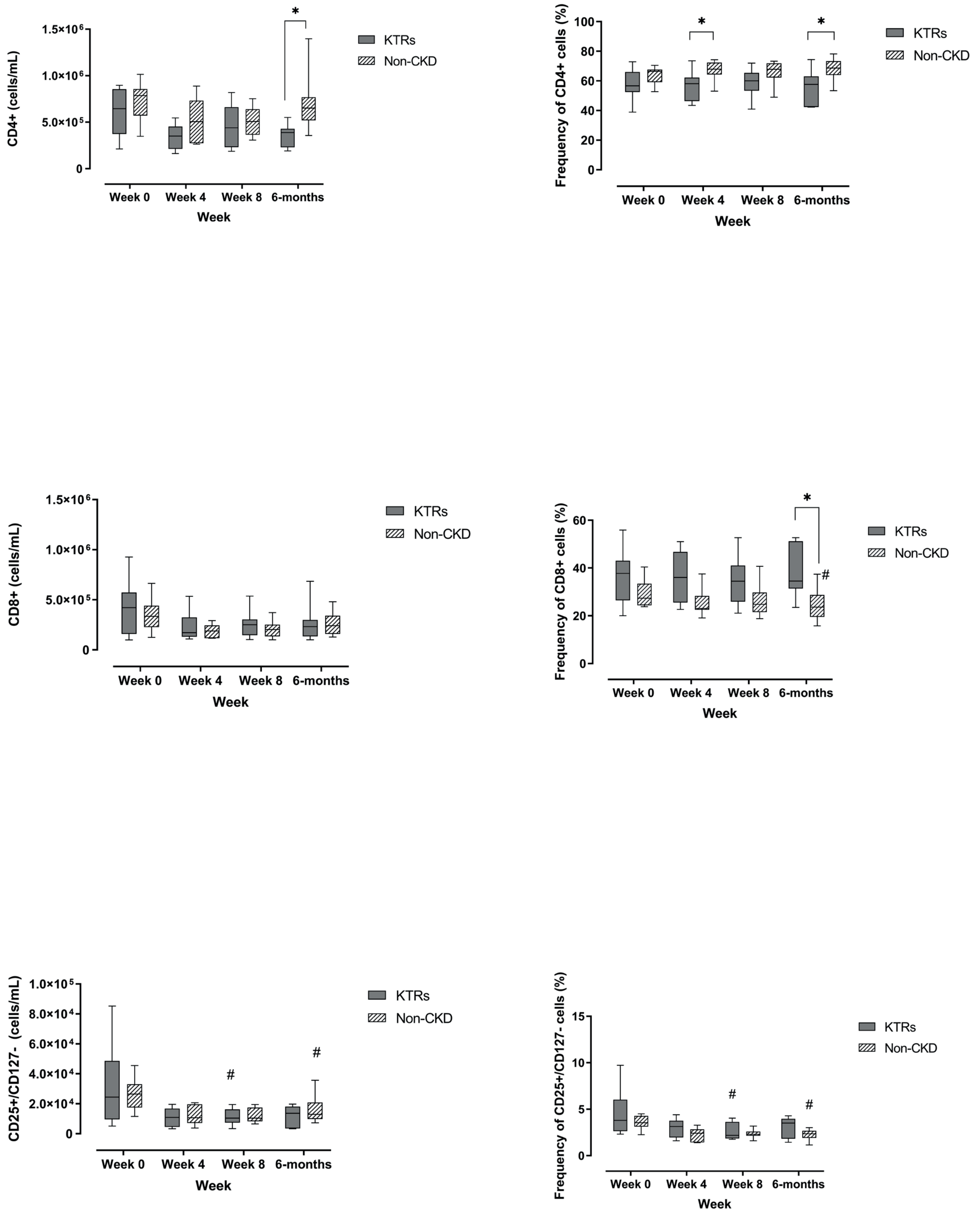


Figure 4. Cell numbers (cells/mL) and cell proportion (%) for CD4+, CD8+, CD25+/CD127- cells at week 0, week 4, week 8 and 6-months for KTRs and Non-CKD controls KTR; kidney transplant recipients, non-CKD; non-chronic kidney disease, non-normally distributed data presented as median and interquartile range. # Denotes significant difference from week 0 for non-CKD and KTRs
* Denotes a significant difference between groups
* Denotes a significant difference between groups, non-CKD greater KTRs

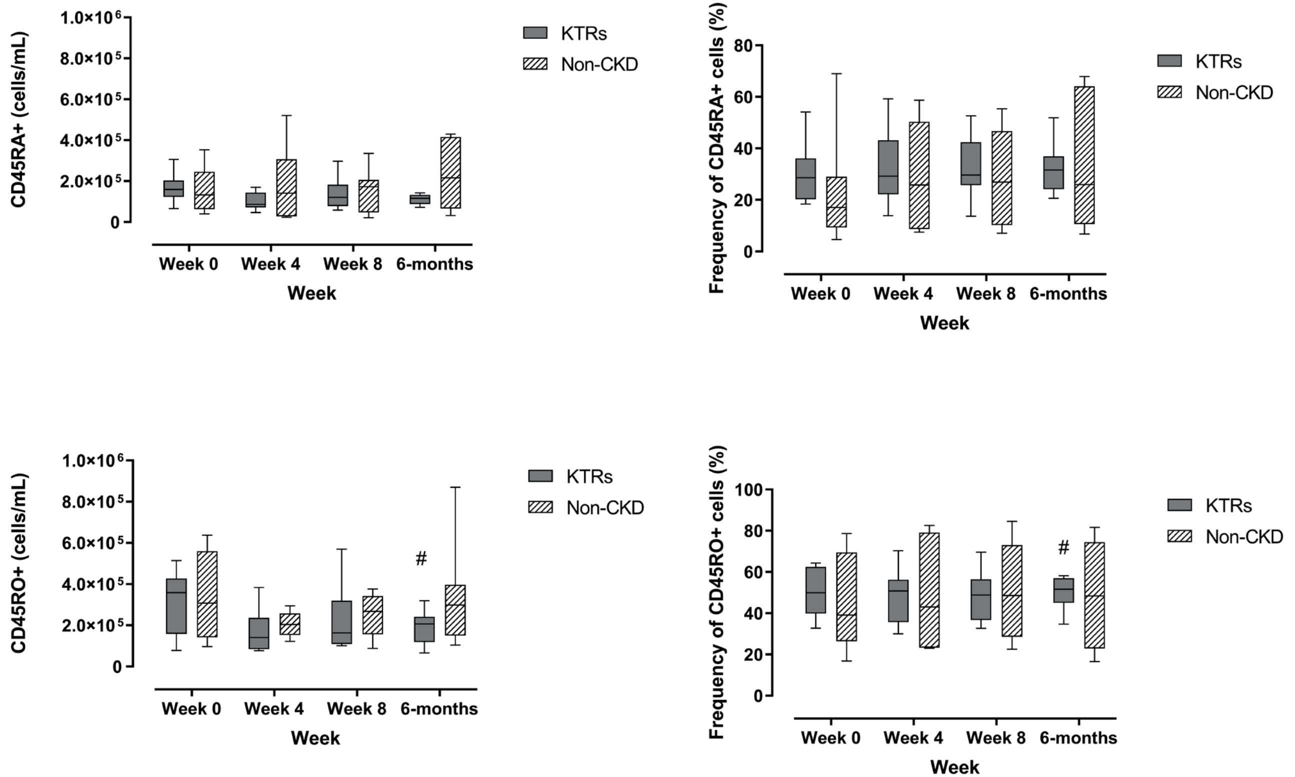


Figure 5. Cell numbers (cells/mL) and cell proportion (%) for CD45RA+, CD45RO+ cells at week 0, week 4, week 8 and 6-months for KTRs and Non-CKD controls KTR; kidney transplant recipients, non-CKD; non-chronic kidney disease, non-normally distributed data presented as median and interquartile range. # Denotes significant difference from week 0 for KTRs
* Denotes a significant difference between groups, non-CKD greater KTRs

ages of CD16+ monocytes compared to healthy controls, initiating an enhanced production of TNF- α, IFN-γ and IL-1β (143), and remaining elevated irrespective of immunosuppressive medications. The present study found no differences in monocyte subsets in stable transplant patients but found elevated concentrations of TNF- α and IL-6. Elevations in TNF-α and IL-6 have been associated with CVD, with reports showing that elevated cytokines is a predictor of death in dialysis patients (144). There have been suggestions that the increase in cytokines could be attributed to either specific renal diseases or other chronic diseases associated with transplantation (145). The KTRs included in this study had been post-transplant 7 ± 5 years, three of these patients suffered from diabetes mellitus, all suffered from hypertension, and one patient suffered from heart disease. These diseases have been associated with inflammation (146, 147). There has also been evidence that greater time post-transplant is associated with increased systemic inflammation and disease (148); however, it is up for discussion whether the inflammation facilitates traditional risk factors for CVD, or whether the CVD itself causes the chronic inflammation. The novelty in the present study findings are that the elevations in cytokine concentrations were independent to monocyte subsets. Further investigation is needed with in vitro analysis to assess cytokine production by monocytes in KTRs and non-CKD controls.

Regular exercise is suggested to be a protective mechanism of CV risk which may indirectly protect against vascular and systemic low-grade inflammation (12, 149). Previous data has reported an anti-inflammatory effect in CKD populations following moderate intensity exercise (121). Reductions in visceral fat mass and increased production of anti-inflammatory cytokines from contracting skeletal muscles are thought the be the mechanisms of the effect (14). During and following exercise, active skeletal muscle increases both cellular and circulating levels of IL-6. The transient rise in circulating IL-6 during exercise appears to be responsible for a subsequent rise in circulating levels of the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist (IL-1RA; (14)), however due to low sample size and lack of plasma samples for acute exercise bouts, we cannot be sure this is true to our patient group.

Our data did not report any changes in long term for circulating cytokines following the exercise intervention. However, higher concentrations of circulating IL-6 and TNF-α were reported in non-exercising transplant patients compared to non-CKD controls. Despite the intensity of the exercise, the study found no evidence for increased chronic inflammation, suggesting HIIT and MICT to not exacerbate inflammation.

A particular side effect of immunosuppressive medication is weight gain (150), given that stable transplant patients are on life-long medications (49) there is a likelihood of increased fat mass. The accumulation of body fat, is associated with increased all-cause mortality (151) and further metabolic syndromes, which is exacerbated through increases in pro-inflammatory adipokines such as TNF-α and IL-6 (10), thus developing a persistent state of low-grade systemic inflammation. Although the present study saw no changes in body fat and body mass following exercise training, there were no further increases in circulating cytokines following 8-weeks of exercise. However, anecdotal evidence did report patients to see a greater weight gain in the subsequent years following

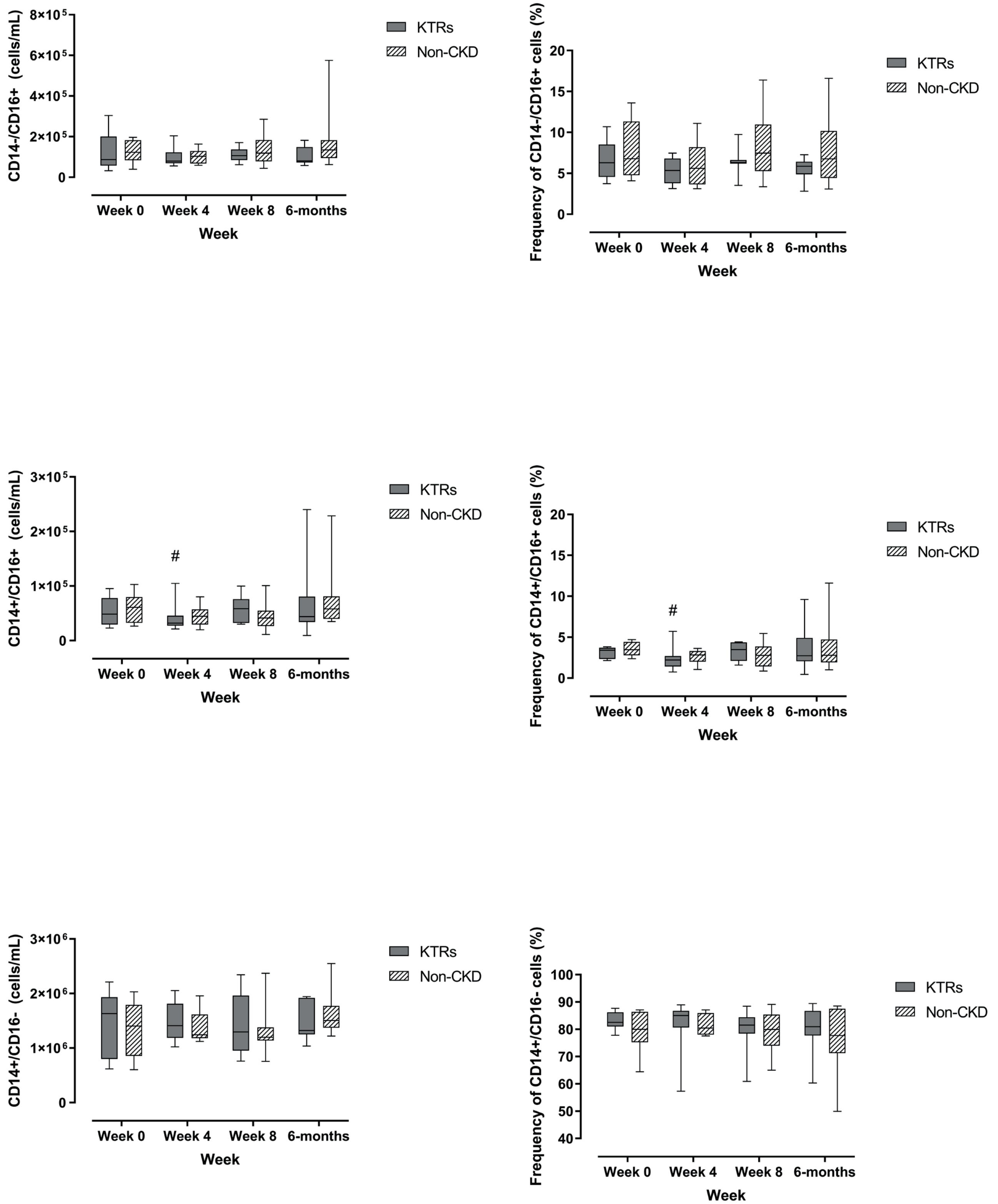


Figure 6. Cell numbers and cell proportion for monocyte subsets at week 0, week 4, week 8 and 6-months for KTRs and non-CKD. KTR; Kidney transplant recipients, non-CKD; non-chronic kidney disease, non-normally distributed data presented as median and interquartile range
# denotes significant difference from week 0 for KTRs

transplantation. It may be plausible that 8-weeks of training may not elicit changes in adipose tissue, but long-term exercise may elicit a reduction in adipose tissues resulting in reductions of circulating pro-inflammatory adipokines as seen in healthy populations.

Circulating immune cells only represent a small part of the immune system. Following exercise, the early inflammatory response is characterised with neutrophils entering the muscle followed by macrophages in regenerating muscle (89, 152). Macrophage-derived growth factors and cytokines are involved in myogenic precursor cell proliferation and differentiation. In animal studies, infusion of TNF-α, IL-1, and IL-6 has led to increased muscle protein breakdown and to muscle atrophy (153). An association between inflammation and muscle wasting has been reported in CKD patients (27). Further to this, reports of elevated mRNA expression of intramuscular IL-6, TNF-α, toll-like receptor-4 (TLR4) and myostatin, while NF-κB and p38 mitogen-activated protein kinase (MAPK) signalling is also upregulated in CKD populations (91, 154) likely resulting in intramuscular inflammation. Inflammation is a regular response to exercise, an 8-week resistance training study in CKD patients (82), reported increases in intramuscular IL-6, MCP-1, and TNF-α within 24 hours after a single exercise bout (155). It may be postulated that following the exercise, intracellular immune and inflammatory cells may be more active than circulating cells, resulting the minimal systemic changes seen in the present study.

4.1.1 Immune cells

Exercise immunology studies have reported transient time-dependant changes to phenotypic and functional capacity of lymphocytes in circulation in response to a single bout of exercise (16). For intense exercise, it is observed that the lymphocyte repertoire is increased during exercise which results in 'stimulation' of the immune system. However, hours following exercise, total lymphocyte numbers decrease to below pre-exercise levels proposing that exercise induces a short-term window of immune suppression. Exercise may deploy immune cells to peripheral tissues (156) , resulting in less mobilisation of immune cells within the circulation. A study by Kruger et al. (2008) found that T cells are redeployed in large numbers to peripheral tissues including the gut and lungs, and to the bone marrow following exercise by using fluorescent cell tracking (157).

4.2 IMPLICATIONS OF RESEARCH

Currently, unified exercise guidelines for KTRs do not exist. Exercise has been incorporated into the International Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines, which suggest some form of aerobic physical activity that is compatible with CV health and tolerance. However, there is a lack of detail for the type, frequency, intensity and duration of the exercise. Recommendations of at least 30 minutes of moderate intensity physical activity, 5 times a week have been suggested for weight management and improvements of CV health and QoL (68), however these studies look more at improving lifestyle factors for individuals with CKD stages 3-5 rather than KTRs (an immunocompromised population). The data in this manuscript suggests that recommending HIIT as a form of exercise is beneficial and can be incorporated into newly formed exercise guidelines for this population.

This project was developed with the help of exercising KTRs who were motivated to do intense exercise but were unsure as to whether it was immunologically safe for them. Given that physiological improvements in HIIT groups were observed without adverse negative effects on immune cells, it may be postulated that that HIIT is safe and tolerable for this population group.

Even though transplantation have been shown to reduce the risk of CV events (3), CVD still remains one of the major causes of mortality in KTRs (158) and outcomes remain poorer than in the general population (4). Chronic inflammation plays a major role in CVD risk (11, 40, 159, 160), and elevations in pro-inflammatory cytokines CRP, TNF-α, IL-6 and IL-1β have been demonstrated to contribute to systemic inflammation (20, 22, 27, 161, 162). In this study, elevations in IL-6 and TNF-α concentrations were reported in non-exercising KTRs compared to their non-CKD counterparts. Other data reports decrease in inflammatory cytokines in heart transplant patients following exercise (163). Given that exercise is suggested to be a therapeutic method to reduce CVD risk factors, and no increases in cytokines were observed following 8-weeks of exercise, HIIT can improve exercise capacity and physical function, but also for reducing CVD risk factors. However, further longitudinal investigations are needed to confirm this.

4.3 STRENGTHS

Given that exercise is becoming more popular with clinical populations for improvements in QoL, the safety and tolerance of different exercise programmes is a major concern. The study PACE-KD presented in this manuscript was designed in response to patient-generated research questions based on exercise performance enhancement and the interaction with immunosuppressive medication.

The flow cytometry panels used to assess the frequency of immune cells are novel panels devised for this study. The immune markers chosen to assess were markers that are suggesting altering from exercise interventions and relevant to kidney populations. The panels and analysis were developed to ensure that any changes in immune cell populations were a result of the exercise intervention rather than deviations of methodologies.

4.4 LIMITATIONS AND FUTURE DIRECTIONS

Some methodological limitations limit the conclusions that can be drawn from the above data. Whilst there were no differences for circulating immune cell subsets, we cannot be sure that these results are the same at the tissue level. Exercise is known to initiate muscle metabolism, therefore the use of muscle biopsies with the intention of immunostaining would generate practical data in addition to what has been presented in this manuscript.

Given that exercise can initiate an immunosuppressive effect in healthy populations (164), and the addition of immunosuppressive medications may initiate further immune suppression, this may result in a window for opportunistic infections. The data presenting in this study reports minimal changes to the frequency of immune cells following a single bout of acute exercise. Future studies may warrant the use of in vitro lymphocyte proliferation assay that would determine whether cells would be triggered to divide after exposure to a specific stimulus e.g. Phytohaemagglutinin P (PHA) or Concanavalin A (Con

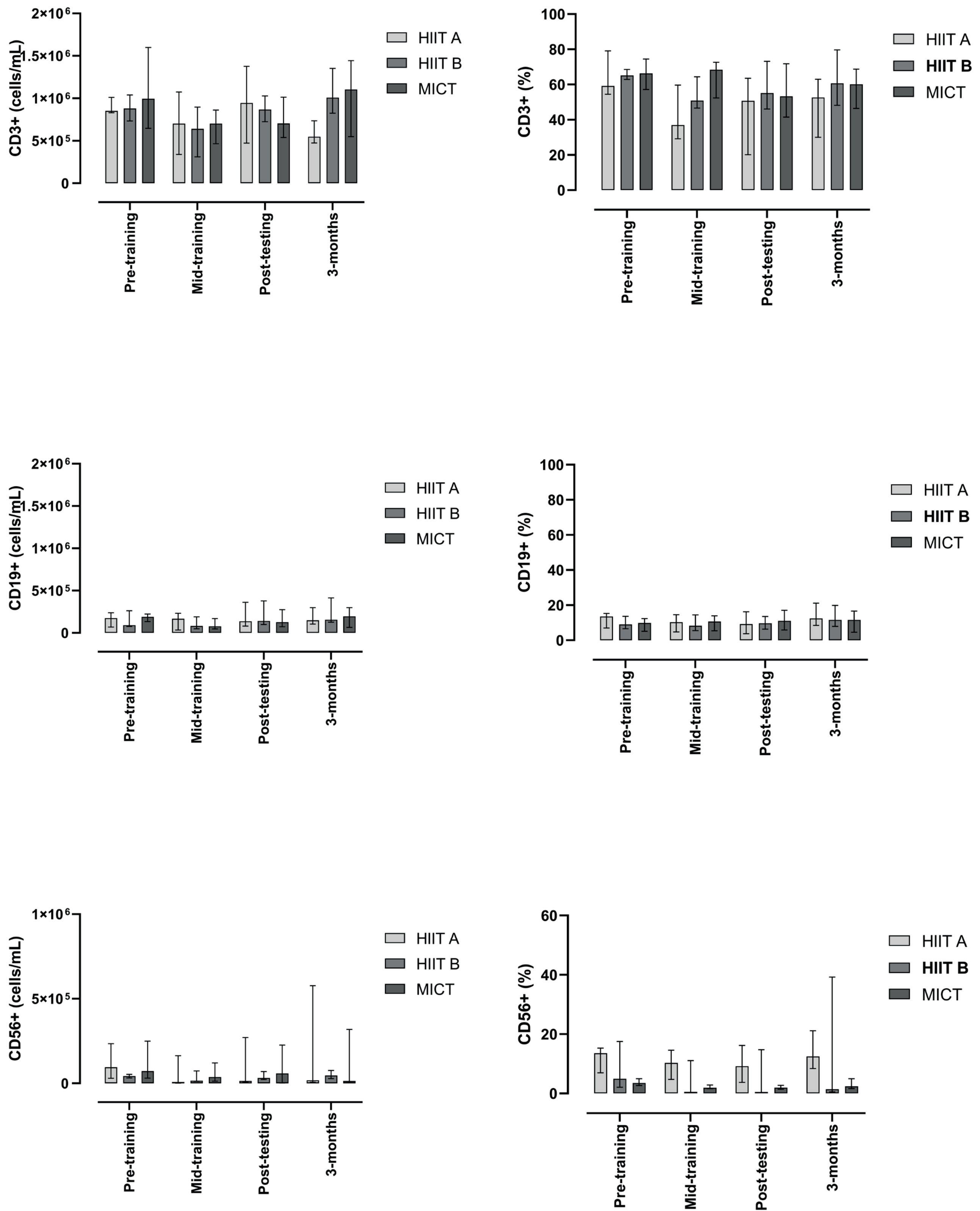


Figure 7. Cell numbers (cells/mL) and cell proportion (%) for CD3+, CD19+, CD56+ cells at pre-training, mid-training, post-training and 3-months post training. HIIT A; high intensity interval training A, HIIT B; high intensity interval training B, MICT; moderate intensity continuous training, non-normally distributed data presented as median and interquartile range

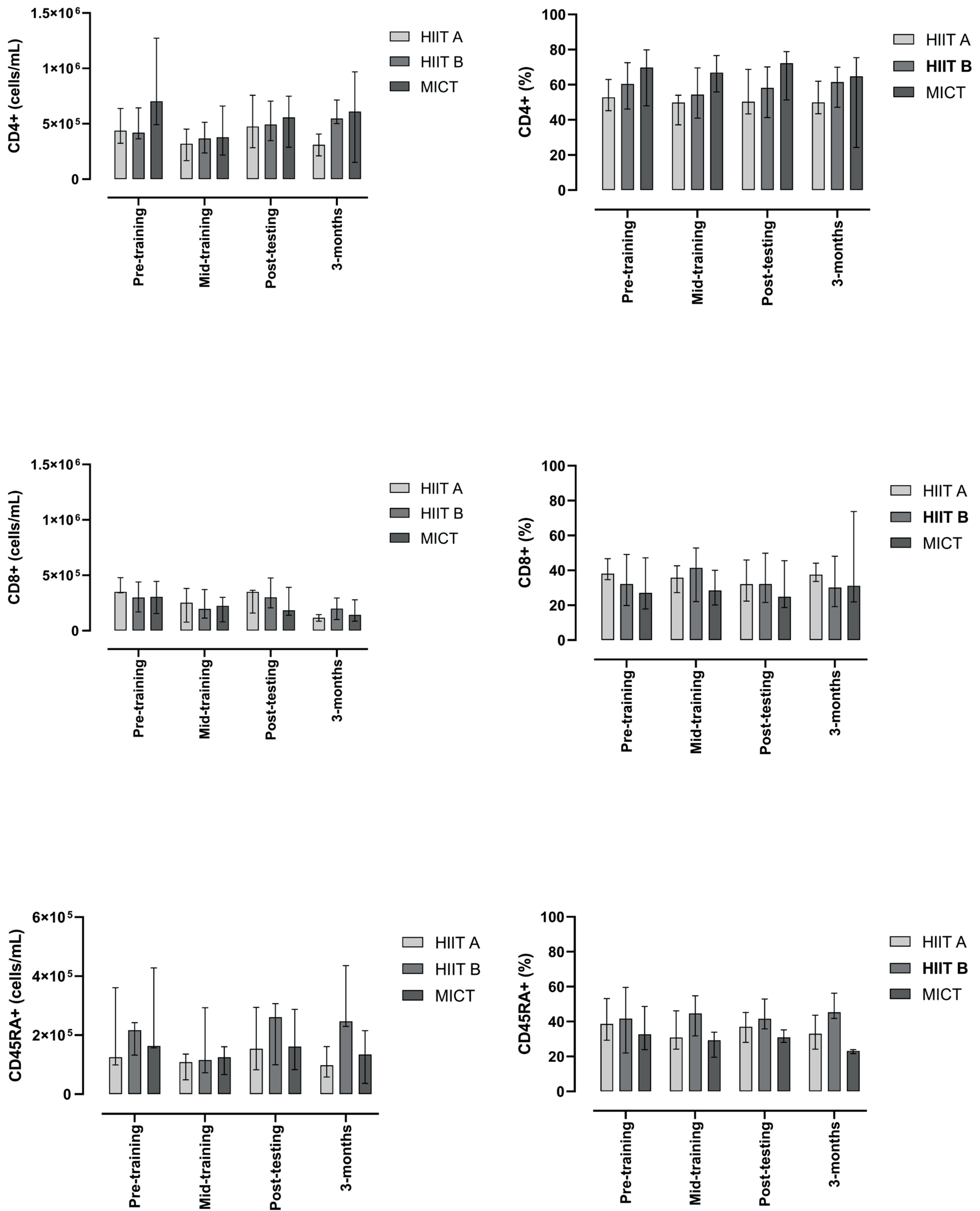


Figure 8. Cell numbers (cells/mL) and cell proportion (%) for CD4+, CD8+, CD45RA+ cells at pre-training, mid-training, post-training and 3-months post training. HIIT A; high intensity interval training A, HIIT B; high intensity interval training B, MICT; moderate intensity continuous training, non-normally distributed data presented as median and interquartile range

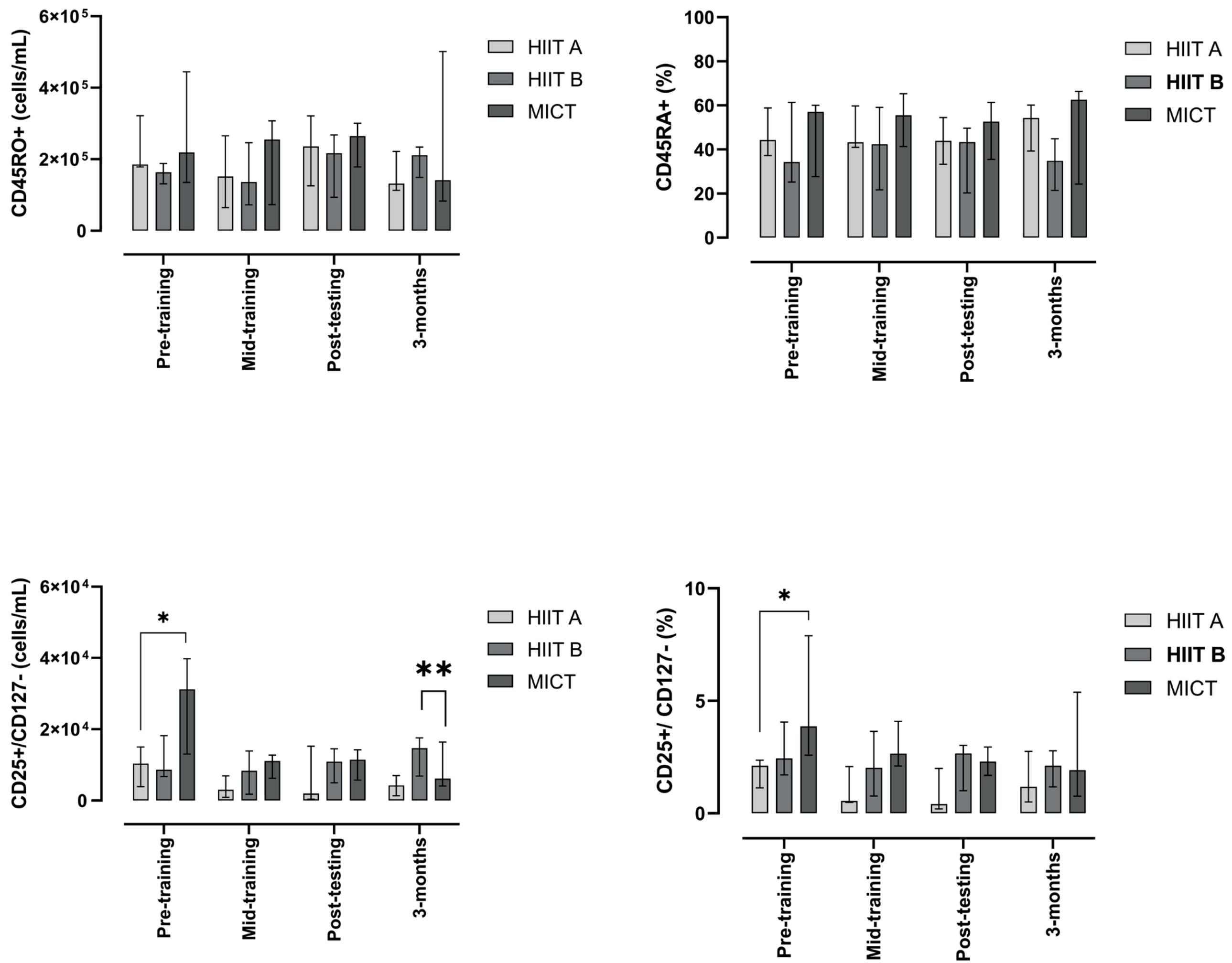


Figure 9. Cell numbers (cells/mL) and cell proportion (%) for CD45RO+, CD25+/CD127- cells at pre-training, mid-training, post-training and 3-months post training.
HIIT A; high intensity interval training A, HIIT B; high intensity interval training B, MICT; moderate intensity continuous training, non-normally distributed data presented as median and interquartile range
* denotes significant difference between groups at pre-training for CD25+ CD127- cells MICT greater than HIIT A
** denotes significant difference between groups at 3-months post training for CD25+ CD127- cells HIIT B greater than MICT

A). This method is quick and inexpensive however the assay would provide information about the health and response of the population of cells as a whole rather than individual cells. The alternative method that would allow information about individual cells is to label cells in culture with a fluorescent marker such as carboxyfluorescein succinamidyl ester (CFSE), which would dilute in each generation of daughter cells as they divide in response to a stimulus and could be measured via multi-colour flow cytometry. Assessing the proliferation and responsiveness of cells would add evidence to the interaction of exercise and immunosuppressive medications on immune cell subsets and may draw some conclusions on timing immunosuppressive medications around exercise, thus advancing the research area.

The HIIT protocols in this study have been used in previous clinical studies (109, 165), with beneficial effects. Following transplantation, exercise adherence is lowered after the first 5 years of transplant (150, 166). The present study found KTRs to attend exercise sessions either before or after work with some patients withdrawing from the study due to other commitments (manuscript under review elsewhere). Therefore, home-based exercise sessions may be more beneficial than hospital-based exercise programmes for greater exercise adherence and physiological benefit (167).

The data presented in this manuscript is from feasibility and pilot studies, therefore small sample size and inadequate detection of statistical changes are seen. However, data can be used to generate sample size calculations for larger trials. Future studies may also want to reduce the number of parameters detected/ time points to prevent spurious significant results.

Finally, exercise has been established as part of a multimodal therapeutic approach to cardiorespiratory fitness, muscle strength, physical function and improvement in quality of life (73). However, the type, duration, intensity of exercise remains obstacles

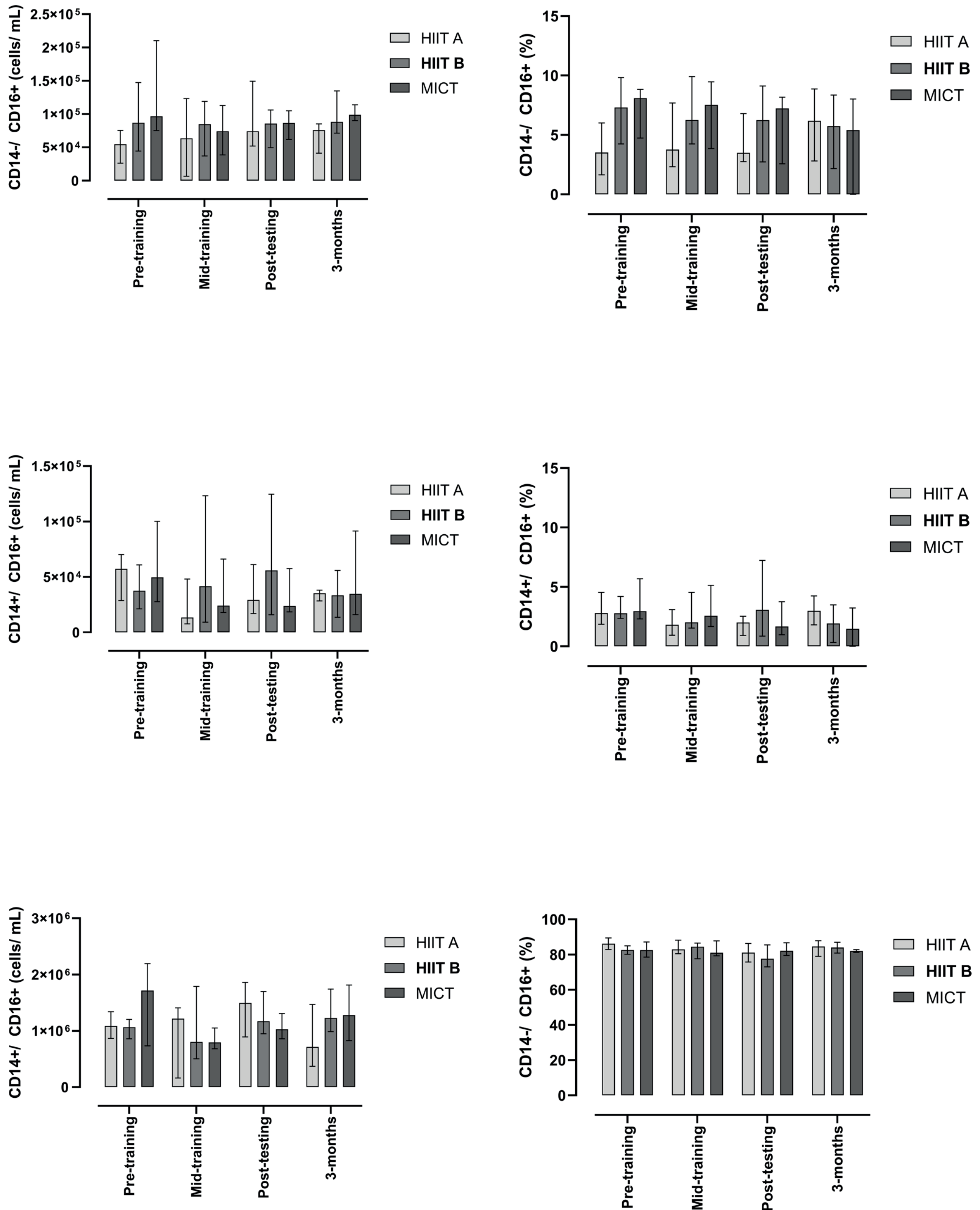


Figure 10. Cell numbers (cells/mL) and cell proportion (%) for monocyte subsets at pre-training, mid-training, post-training and 3-months post training.
HIIT A; high intensity interval training A, HIIT B; high intensity interval training B, MICT; moderate intensity continuous training, non-normally distributed data presented as median and interquartile range

in the assessment of measurable effects of exercise on inflammatory markers. This paper demonstrates that regular exercise may not have any negative effects on immune parameters in KTRs. As exercise helps to improve CV health, regular exercise should be encouraged following transplantation as it appears to have a physiological benefit. This manuscript is one of first forms of research to explore HIIT on immune cells of immunocompromised patients. Muscle metabolism studies are needed using non-exercising KTRs, exercising KTRs and healthy controls for the assessment of mitochondrial oxidative capacity and fluorescent immune cell trafficking within the muscle. This would allow researchers to investigate whether CKD and transplant populations are able to make physiological adaptations or initiate a training response. Inhibition of protein synthesis, increased protein catabolism and mitochondrial impairment leads to weakness and there is some evidence that immunosuppressant drugs may potentiate one another in their detrimental effects on skeletal muscle (168, 169).

Unanswered questions remain for the immunological consequences of HIIT and the interaction of immunosuppressive medications for the infection risk and graft rejection of this population. To investigate infection risk and graft rejection, firstly, a proliferative assay with an unspecific or specific stimulus in culture would identify how the cells response before and after the exercise interventions. Secondly, treating the cell cultures with an immunosuppressive reagent before and subsequently after exercise, would allow some conclusions to be drawn about the optimum timing of immunosuppressant regimes.

4.5 CONCLUDING REMARKS

CVD, infection, and malignancy are common in KTRs. Exercise can alter immunity. This study demonstrated that 8-weeks of HIIT and MICT programmes presented in this study did not cause any immediate adverse negative effects on immunity. KTRs can exercise at a range of high intensities without worrying about long-term alterations to immune parameters., however there must be caution when working at greater intensities than presented here.

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AUTHORSHIP:

A.C.S. and N.C.B. contributed to the research idea and study design. G.M.H., A.M.C., R.E.B., and D.G.D.N. contributed to the generation/collection of data. G.M.H. and A.M.C. were involved in data interpretation. A.M.C. and A.C.S. were involved in supervision. G.M.H. drafted the manuscript. Each author contributed important intellectual content during manuscript revision and accepts responsibility for the overall work.

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Exercise effect on symptom severity, morbidity and mortality in viral infections: a systematic review and meta-analysis.

Rafaela Bertini de Araujo[a], Mara Patrícia Traina Chacon-Mikahil[a,b], Janet M. Lord[c,d], Amanda Veiga Sardeli[a,b, c, d]

[a] Laboratory of Exercise Physiology (FISEX), School of Physical Education, University of Campinas, Campinas, Brazil.
[b] Graduate Program in Gerontology, Faculty of Medical Sciences, University of Campinas, Campinas, Brazil.
[c] MRC-Versus Arthritis Centre for Musculoskeletal Ageing Research, Institute of Inflammation and Ageing, University of Birmingham, Birmingham, UK.
[d] NIHR Birmingham Biomedical Research Centre, University Hospital Birmingham and University of Birmingham, Birmingham, UK.

ABSTRACT

There is a knowledge gap regarding the consequences of exercise during acute infections in humans and contradictory findings in animal studies, compromising public health advice on the potential benefits of physical activity for immunity. Here, we carried out a meta-analysis of studies of the effects of moderate exercise (ME) and exercise until fatigue (EF) on symptom severity, morbidity and mortality during viral infection in animal models. The systematic review on PubMed, Scopus, Embase, Web of Science, Cochrane and EBSCOhost (CINAHL and SPORT Discus) identified 8 controlled studies, with 15 subgroups within them. The studies exposed the animals (mice [7 studies] and monkeys [1 study]) to exercise immediately before or after viral inoculation (HSV-1, H1N1 influenza and B.K. virus) with follow-up for 21 days. ME significantly reduced morbidity (OR 0.43 [0.19; 0.98], P = 0.04) with no change for symptom severity (SMD -3.37 [-9.01; 2.28], P = 0.24) or mortality (OR 0.48 [0.08;3.03], P = 0.43). In contrast, EF gave a trend towards increased symptom severity (SMD 0.96 [-0.06; 1.98], P = 0.07) and mortality (OR 1.47 [0.96;2.28], P =0.08) with no change in morbidity (OR 1.22 [0.60;2.5], P = 0.58). We conclude that in animals moderate exercise during infection is advantageous, whilst exercise until fatigue should be avoided. Further research is required to determine if moderate exercise may also be beneficial in humans during infection.

Keywords: Immunity, Virus Infection, Physical Activity, Exercise, Survival.

Corresponding author:
Amanda Veiga Sardeli, Institute of Inflammation and Ageing, Queen Elizabeth Hospital, Mindelsohn Way, Birmingham, West Midlands, B15 2WB, UK, E-mail: a.veigasardeli@bham.ac.uk.

INTRODUCTION

There is a considerable literature supporting the benefits of exercise for human health in general (10) and for the immune system in particular (7, 16, 21). The positive effects of exercise include an enhanced response to vaccination (39), improved immune surveillance mediated by redistribution of immune cells to tissues following exercise (30, 52), increased apoptosis of senescent T cells potentially rejuvenating the immune system (33, 51); and with maintained physical activity in to old age there is evidence that the negative effects of age upon immune phenotype and immune responses can be reduced (17, 46). Regards effect of exercise on occurrence, severity and duration of acute respiratory infections, a comprehensive meta-analysis showed exercise reduced the severity of symptoms and the number of symptom days (22). However, the benefits of exercise during an acute infection have received less attention and some of the data concerning the immune response to acute exercise, such as temporary lymphopenia (45, 49, 50) and reduced salivary immunoglobulin A levels (36), have been interpreted as indicating immune suppression (27). Whilst alternative interpretations of the exercise immunology literature have been made (7), recommendations for conservative exercise protocols, and even exercise restriction at times of infection persist (20, 26). Crucially, as there is also good evidence that exercising skeletal muscle is a major positive regulator of immune function (3, 16), it is also possible that exercise could enhance the immune response against viruses and bacteria and reduce the burden of latent viral infections (1, 22, 54). In the absence of infection, exercising skeletal muscle is the major producer of a range of cytokines including IL-6 which in this context has anti-inflammatory actions (3), for example induction of IL-10, and IL-1RA production by macrophages (40). Muscle also produces cytokines such as IL-7 and IL-15 which support the function of the thymus and enhance the survival and function of immune cells (24, 43).

Opinions, reviews and practical guidelines discussing the risk of acute exercise during infection in humans, including the recent COVID-19 pandemic, are based largely on indirect evidence (18, 20, 57, 59) and only two controlled trials in humans have been reported that directly tested exercise effects on symptom severity during infections (55, 56). In the first, rhinovirus 16 was inoculated into moderately fit young adults who then underwent 40 minutes of aerobic exercise at 70% of their reserve heart rate for the following 7 days (56). There was no difference for the number of symptoms or the symptom severity measure between the exercise and control groups during the 10 day follow-up (56). However in this study symptom severity was assessed only by weighing the mucus (nasal secretion) instead of a more robust and/or sensitive method to

confirm the duration of infection (56). A second study by the same group assigned non-physically active young adults with a naturally acquired upper respiratory tract infection to either 30 minutes aerobic exercise at 70% of target heart rate for five days, or control period with no exercise (55). The study found no difference between mean symptom score and mean number of days with symptoms (55). Although these studies do not make a case for the benefits of exercise during infection, in healthy young individuals, they also do not support the school of thought that exercise is detrimental at times of infection.

In contrast to the lack of human studies, studies in animals, including mice and primates, have considered the influence of exercise on infection outcomes (11, 31). These studies have shown negative effects of exercise carried out before virus inoculation, with increased symptom severity, morbidity and mortality in mice and monkeys compared to controls (11, 31). These apparent differences in outcome between humans and animals could be caused by the differences in volume and intensity of exercise performed in animals, as some studies have used exercise with exhaustive protocols (11). In fact, after a marathon race, humans undergo some reduction in delayed-type hypersensitivity response, salivary IgA, T cell function, NK cell activity, macrophage function, granulocyte oxidative burst together with increase in neutrophil/lymphocyte ratio, cytokines and stress hormones that would lead to transient immune dysfunction (37).

To address the discordance between studies and attempt to come to some consensus regarding exercise and the response to viral infections, we aimed to carry out a meta-analysis of the effects of moderate exercise (ME) and exercise until fatigue (EF) on symptom severity, morbidity and mortality during viral infection in animal studies.

METHODS

All details of the review protocol can be seen on PROSPERO (CRD42021277401). We searched on PubMed (MEDLINE), adapted to Embase Scopus, Web of Science, Cochrane, and EBSCOhost, April 19, 2021, for controlled studies testing the effect of any type of exercise in animals during infection. They could be purposely or naturally exposed to infection by any virus, and they needed to report the impact on morbidity, symptom severity, or mortality in the exercise and control groups. Duplicates were automatically removed using the Mendeley reference manager system and the selection of studies was done by two independent reviewers on the Rayyan-Systematic Reviews system (38).

Morbidity was assessed as the percentage of sick animals on the last day of follow up which was at day 21; symptom severity was assessed by scales that would consider different symptoms such as ruffled fur, inactivity, hunched back, and redness around eyes, nose, or mouth; mortality was assessed by percentage of deaths during the study period. Thus, morbidity and mortality were assessed as odds ratio (OR and 95% CI), according to the following equation OR = (n events in EXERCISE / n total in EXERCISE) / (n events in CONTROL/ n total in CONTROL). The symptom severity was calculated as the standardized mean difference (SMD) and 95% CI between exercise and control means at a given day.

The three meta-analyses were performed using Comprehensive Meta-Analysis software, version 3.3.070. When there was statistical significance for heterogeneity, randomized effect models were selected and when there was no significant heterogeneity, fixed effects were applied. The inconsistency between studies was reported as a percentage (I^2), based on difference between expected heterogeneity (df) and true heterogeneity (Q-value). The subgroups within studies were clustered according to exercise protocols performed until fatigue (EF) or protocols of moderate intensity

Table 1. Characteristics of the studies included.

First Author, Year	Species	Age	Sex		Exercise time-point	Intensity category/ protocol	Type	Volume/Duration	Morbidity	Mortality	Symptoms Severity
Levinson, 1945 (31)	Monkeys (Macaca mulatta)	NR	M/F	BKV (intracerebrally)	Post inoculation	EF/ Fatigue	Swimming	2-3 hours/ 4 d	Yes (days 11-14)	-	Yes *
Davis,1997 (11)	Mice	4 wk	M	HSV-1 (Intranasal)	Before inoculation	EF/ Fatigue	Running (treadmill)	2.5–3.5 hours/ 3 d	Yes (day 21);	Yes (day 21)	-
						ME/ NR	Running (treadmill)	30 minutes/ 3 d			
Brown, 2004 (6)	Mice	~60 d	M/F	HSV-1 (Intranasal)	Before inoculation	EF/ 70–80% V̇O2 max.	Running (treadmill)	135 ± 5 min/ 3 d	Yes (day 21)	Yes (day 21)	Yes (1° day of symptom)
Davis, 2004 (12)	Mice	4 wk	M	HSV-1 (Intranasal)	Post inoculation	ME/ 68-78% V̇O2 max.	Running (treadmill)	1 hour/ 6 d	Yes (day 21);	Yes (day 21)	
Murphy, 2004 (34)	Mice	4 wk	M	HSV-1 (Intranasal)	Post inoculation	ME/ 75-90% V̇O2 max.	Running (treadmill)	1 hour/ 6 d	Yes (day 21);	Yes (day 21)	Yes (day 7)
Lowder, 2005 (32)	Mice	20-24 wk	M	H3N2 (Intranasal)	Post inoculation	EF/ 65-70% V̇O2 max.	Running (treadmill)	2.5 hours/ 3 d;	Yes (day 21);	Yes** (day 21)	
						ME/ 65-70% V̇O2 max.	Running (treadmill)	30 min/ 3 d			
Brown, 2007 (5)	Mice	7 wk	F	HSV-1 (Intranasal)	Before inoculation	EF/ 70–80% V̇O2 max.	Running (treadmill)	20 min/ 3 d,	Yes (day 21);	Yes (day 21)	Yes (days 12, 16-21)
Murphy, 2008 (35)	Mice	4 wk	M	H1N1 (Intranasal)	Post inoculation	EF/ 70–80% V̇O2 max.	Running (treadmill)	20 min/ 3 d	Yes (day 21);	Yes (day 21)	Yes (day 7)

Legend: BKV: BK virus; d: days; EF: Exercise-fatigue; ME: moderate exercise; F: Female; H1N1: Influenza A virus subtype H1N1; HSV-1: herpes simplex virus 1; M: Male; Min: Minutes; NR: Not report; $VO_{2\,max}$ refers to the maximum amount of oxygen you can utilize during exercise; wk: weeks;* Assessed by incidence of paralysis and degree of involvement (not included in the meta-analysis); **The animals were followed up for 30 days, but the 21th day was meta-analysed in order to maintain consistency between studies.

(ME). Although, one of the studies described its exercise group as prolonged exercise, it was analyzed as EF (32). Q tests were applied to group comparisons, considering 95% confidence. Egger's tests were performed to check the risk of publication bias in each meta-analysis.

RESULTS

Supplementary figure 1 details the flowchart of selection of studies that led to inclusion of 8 controlled studies, with 15 subgroups within them. The characteristics of the studies are summarized on table 1.

The meta-analysis of morbidity included 249 animals in the EXERCISE and 393 animals in the CONTROL. Since some studies had more than one group of intervention and control (e.g.: males and females), each controlled intervention was included as a separated study for analysis (4, 6, 31, 34). The overall hypothesis test showed the meta-analysis was not significant (OR 0.90 [0.46; 1.77], P = 0.77), with significant heterogeneity and inconsistency across studies ($P < 0.001$; $I^2 = 67.94\%$), and significant risk of bias (Egger test, P = 0.02). Figure 1a shows EF did not alter morbidity compared to CONTROL (OR 1.22 [0.60;2.5], P = 0.58), while ME significantly reduced morbidity in comparison to CONTROL (OR 0.43 [0.19; 0.98], P = 0.04).

The meta-analysis of symptom severity included 178 animals in the EXERCISE and 182 animals in the CONTROL. The days that each study reported the severity peak of symptoms were included for analysis, except for one study that reported the first day of symptoms rather than its severity peak (6). The overall hypothesis test showed there was no significant difference between the EXERCISE and CONTROL groups (SMD 0.05 [-1.04; 1.14], P = 0.93), with significant heterogeneity and inconsistency across studies ($P < 0.001$; $I^2 = 94.66\%$), and non-significant risk of bias (Egger test, P = 0.75). Figure 1b shows EF trended towards a higher severity of symptoms compared to CONTROL (SMD 0.96 [-0.06; 1.98], P = 0.07), with no difference between ME and CONTROL (SMD -3.37 [-9.01; 2.28], P = 0.24).

The meta-analysis of mortality included 371 animals in the EXERCISE and 370 animals in the CONTROL. The overall hypothesis test showed the meta-analysis was not significant (OR 1.07 [0.51; 2.21], P = 0.17), with significant heterogeneity and inconsistency across studies ($P < 0.001$; $I^2 = 76.31\%$), and non-significant risk of bias (Egger test, P = 0.94). Figure 1c shows EF trended towards higher mortality than CONTROL (OR 1.47 [0.96; 2.28], P = 0.08), while ME was no different from CONTROL (OR 0.48 [0.08; 3.03], P = 0.43).

SYRCLES`s risk of bias tool (25) showed low quality within the primary studies, in which the large majority of them did not report whether group allocation was adequately concealed, whether caregivers and outcome assessors were blinded; whether the animals were selected at random for outcome assessment, and incomplete outcome were not reported (Supplementary Table 1). At last, there was low quality of evidence (score 2) for the severity of symptoms and Mortality meta-analyses, whilst there was very low quality of evidence (score 1) for the morbidity meta-analysis assessed by the GRADE approach (23). In summary, the three meta-analyses lost two points due to its considerable inconsistency and low quality in their primary studies (score between 4 and 5 on SYRCLES); only the morbidity meta-analysis lost one more point due to its significant risk of publication bias; and all three led to precise results by direct evidence.

DISCUSSION

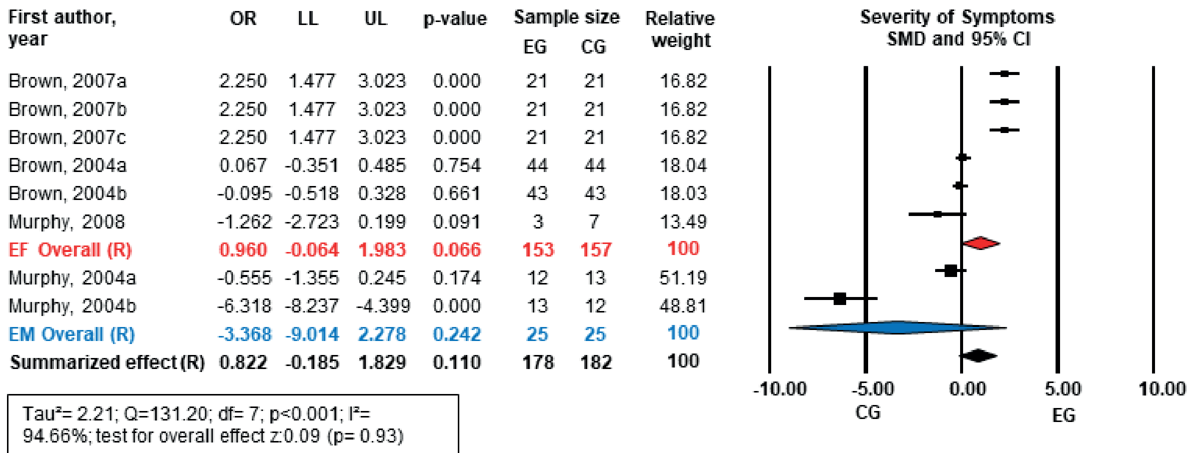
Eichner (18) first questioned why someone should exercise during an infection if the workout intensity will be suboptimal to increase performance or skills. However, the loss of strength, muscle mass, and cardiorespiratory capacity are remarkable after a few days of de-training, such as during bed rest with or without an infection (2, 13, 42). An argument therefore could be made for maintaining exercise routines during an infection to avoid deconditioning. This may be even more important in older individuals who are already at increased risk of sarcopenia and frailty (48, 58). Older adults also have compromised immune systems which increase their risk of infections and of succumbing to more severe symptoms, as demonstrated in the COVID-19 pandemic (19).

Here we showed that moderate exercise could be a tool to boost immune responses as we found a significant reduction in morbidity in animal studies of viral infection using such exercise programmes. Many physiological mechanisms could be mediating such benefits. Acute exercise sessions repeated over several weeks increase antibody production and cell-mediated responses during vaccination (39) and transiently enhance immune system features such as reducing the number of senescent lymphocytes in the circulation (29, 44), increasing in blood counts for neutrophils, lymphocytes, monocytes, and natural killer cells (37). Through the increase in cortisol and adrenaline, and possibly also increased blood and lymph circulation, exercise stimulates leukocyte circulation, release of cytokines, chemokines, in turn facilitating antigen recognition, processing, and presentation, as well as cell migration to lymph nodes and cell differentiation (39, 41).

In contrast, we found that exercise to fatigue trended towards an increase in the severity of symptoms and mortality. The exact mechanism that explains the differences between types of exercise are unknown. However, the exercise to fatigue could affect different pathways that contribute to reduced immune responses. For example, the generation of Damage Associated Molecular Patterns (DAMPs) from damaged muscle which is then recognized by TLR receptors and could lead to immune paresis (8, 28). Production of immune suppressive stress hormones such as cortisol would also impact on immunity and reduction in energy availability with these longer duration exercise protocols could compromise lymphocyte proliferation which is highly energy dependent (15, 37, 47). It is worth noting that animals who are forced to perform exercise would be more stressed than during voluntary exercise, which would trigger a negative immune response (9, 14, 53).

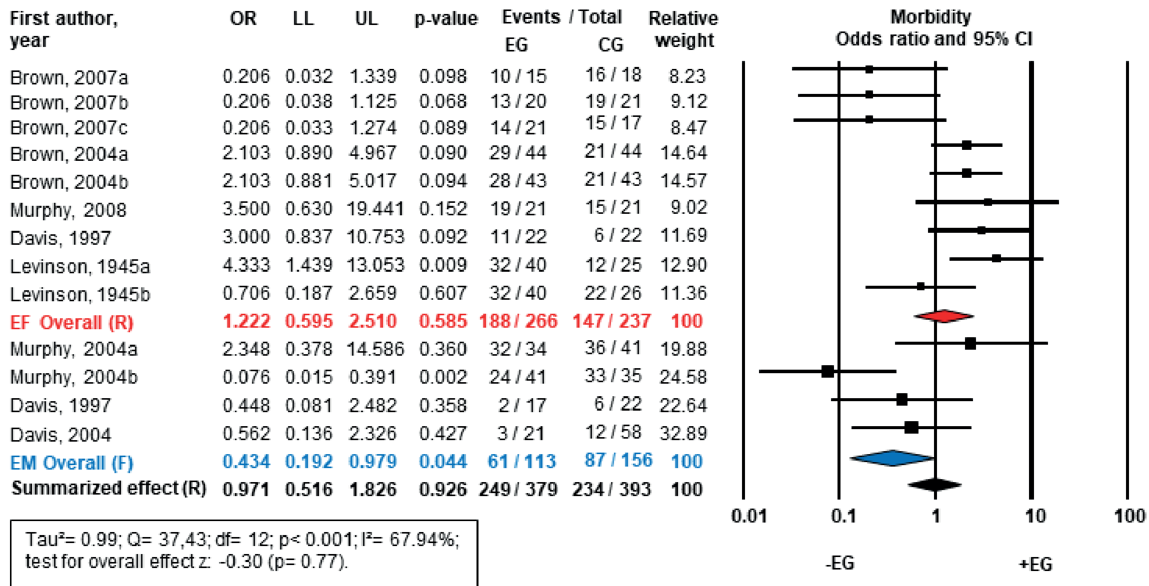
Considering that the studies in the meta-analysis were performed in previously healthy, young animals, the effect of exercise during infections in a high-risk population such as older animals or humans remains to be determined. The only two studies testing exercise effects during infection in humans were in healthy young adults but showed that moderate exercise did not alter symptom severity (55, 56). As these adults would have

Figure 1. Forest plots of the effect of acute exercise on symptom severity (a), morbidity (b) and mortality (c) during acute virus infections. CG: control group; CI: confidence interval; df: degrees of freedom; EG: exercise group; F: fixed effect; I²: percentage of inconsistency between studies; LL: Lower limit; OR: Odds ratio; PBS: PBS liposomes; Q: true heterogeneity; R: random effect; SMD: standardized mean difference; UL: Upper limit; Brown, 2007a: intact (Sham) group; Brown, 2007b: ovariectomized group; Brown, 2007c: ovariectomized and estrogen-supplemented group; Brown, 2004a: female group; Brown, 2004b: male group; Levinson, 1945a: Cage control group; Levinson, 1945b: Water control group; Murphy, 2004a: clodronate encapsulated liposomes intranasally administered group; Murphy, 2004b: PBS liposomes intranasally administered group.



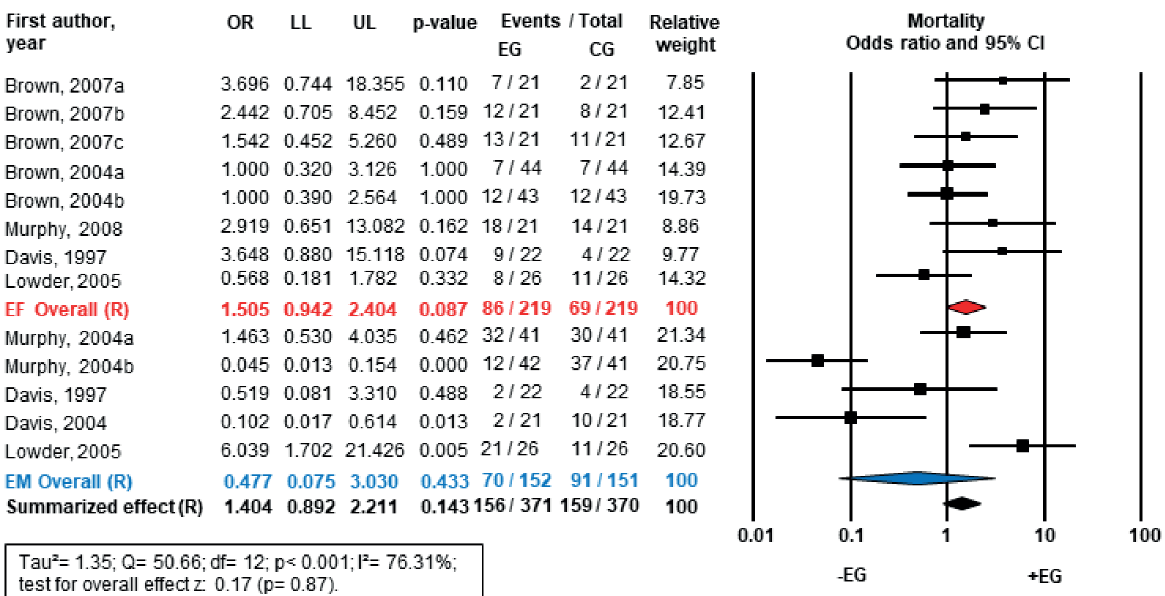
First author, year	OR	LL	UL	p-value	Sample size EG	Sample size CG	Relative weight
Brown, 2007a	2.250	1.477	3.023	0.000	21	21	16.82
Brown, 2007b	2.250	1.477	3.023	0.000	21	21	16.82
Brown, 2007c	2.250	1.477	3.023	0.000	21	21	16.82
Brown, 2004a	0.067	-0.351	0.485	0.754	44	44	18.04
Brown, 2004b	-0.095	-0.518	0.328	0.661	43	43	18.03
Murphy, 2008	-1.262	-2.723	0.199	0.091	3	7	13.49
EF Overall (R)	0.960	-0.064	1.983	0.066	153	157	100
Murphy, 2004a	-0.555	-1.355	0.245	0.174	12	13	51.19
Murphy, 2004b	-6.318	-8.237	-4.399	0.000	13	12	48.81
EM Overall (R)	-3.368	-9.014	2.278	0.242	25	25	100
Summarized effect (R)	**0.822**	**-0.185**	**1.829**	**0.110**	**178**	**182**	**100**

Tau²= 2.21; Q=131.20; df= 7; p<0.001; I²= 94.66%; test for overall effect z:0.09 (p= 0.93)



First author, year	OR	LL	UL	p-value	Events / Total EG	Events / Total CG	Relative weight
Brown, 2007a	0.206	0.032	1.339	0.098	10 / 15	16 / 18	8.23
Brown, 2007b	0.206	0.038	1.125	0.068	13 / 20	19 / 21	9.12
Brown, 2007c	0.206	0.033	1.274	0.089	14 / 21	15 / 17	8.47
Brown, 2004a	2.103	0.890	4.967	0.090	29 / 44	21 / 44	14.64
Brown, 2004b	2.103	0.881	5.017	0.094	28 / 43	21 / 43	14.57
Murphy, 2008	3.500	0.630	19.441	0.152	19 / 21	15 / 21	9.02
Davis, 1997	3.000	0.837	10.753	0.092	11 / 22	6 / 22	11.69
Levinson, 1945a	4.333	1.439	13.053	0.009	32 / 40	12 / 25	12.90
Levinson, 1945b	0.706	0.187	2.659	0.607	32 / 40	22 / 26	11.36
EF Overall (R)	1.222	0.595	2.510	0.585	188 / 266	147 / 237	100
Murphy, 2004a	2.348	0.378	14.586	0.360	32 / 34	36 / 41	19.88
Murphy, 2004b	0.076	0.015	0.391	0.002	24 / 41	33 / 35	24.58
Davis, 1997	0.448	0.081	2.482	0.358	2 / 17	6 / 22	22.64
Davis, 2004	0.562	0.136	2.326	0.427	3 / 21	12 / 58	32.89
EM Overall (F)	0.434	0.192	0.979	0.044	61 / 113	87 / 156	100
Summarized effect (R)	**0.971**	**0.516**	**1.826**	**0.926**	**249 / 379**	**234 / 393**	**100**

Tau²= 0.99; Q= 37,43; df= 12; p< 0.001; I²= 67.94%; test for overall effect z: -0.30 (p= 0.77).



First author, year	OR	LL	UL	p-value	Events / Total EG	Events / Total CG	Relative weight
Brown, 2007a	3.696	0.744	18.355	0.110	7 / 21	2 / 21	7.85
Brown, 2007b	2.442	0.705	8.452	0.159	12 / 21	8 / 21	12.41
Brown, 2007c	1.542	0.452	5.260	0.489	13 / 21	11 / 21	12.67
Brown, 2004a	1.000	0.320	3.126	1.000	7 / 44	7 / 44	14.39
Brown, 2004b	1.000	0.390	2.564	1.000	12 / 43	12 / 43	19.73
Murphy, 2008	2.919	0.651	13.082	0.162	18 / 21	14 / 21	8.86
Davis, 1997	3.648	0.880	15.118	0.074	9 / 22	4 / 22	9.77
Lowder, 2005	0.568	0.181	1.782	0.332	8 / 26	11 / 26	14.32
EF Overall (R)	1.505	0.942	2.404	0.087	86 / 219	69 / 219	100
Murphy, 2004a	1.463	0.530	4.035	0.462	32 / 41	30 / 41	21.34
Murphy, 2004b	0.045	0.013	0.154	0.000	12 / 42	37 / 41	20.75
Davis, 1997	0.519	0.081	3.310	0.488	2 / 22	4 / 22	18.55
Davis, 2004	0.102	0.017	0.614	0.013	2 / 21	10 / 21	18.77
Lowder, 2005	6.039	1.702	21.426	0.005	21 / 26	11 / 26	20.60
EM Overall (R)	0.477	0.075	3.030	0.433	70 / 152	91 / 151	100
Summarized effect (R)	**1.404**	**0.892**	**2.211**	**0.143**	**156 / 371**	**159 / 370**	**100**

Tau²= 1.35; Q= 50.66; df= 12; p< 0.001; I²= 76.31%; test for overall effect z: 0.17 (p= 0.87).

highly functional immune systems, the benefits of exercise may be more marked in an older population with compromised immunity (16).

The main limitation of this study was the high inconsistency and low quality of evidence in each analysis suggesting that more studies will be necessary to identify the potential causes of heterogeneity between studies. Since most of the analyses were heterogeneous, we believe the difference between studies might be caused by a variety of factors such as: type and dose of pathogen; the mode, duration and intensity of exercise; and timing of virus administration in relation to exercise treatment.

Another potential limitation was the inclusion of two exercise interventions to fatigue in monkeys (31) instead of mice in the meta-analysis assessing morbidity. However, we ran a separate analysis without these interventions and confirmed the same results as the analysis with all studies included (OR 1.056 [0.448; 2.489], P = 0.901)

In conclusion, while exercise to fatigue trended to increase symptom severity and mortality during infections in animals, moderate exercise did not and significantly reduced mortality. Future studies should test the effect of moderate intensity exercise during infections in humans as a potential therapy to reduce symptom burden and accelerate recovery.

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Statement of Ethics
An ethics statement is not applicable as this study is based exclusively on published literature.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

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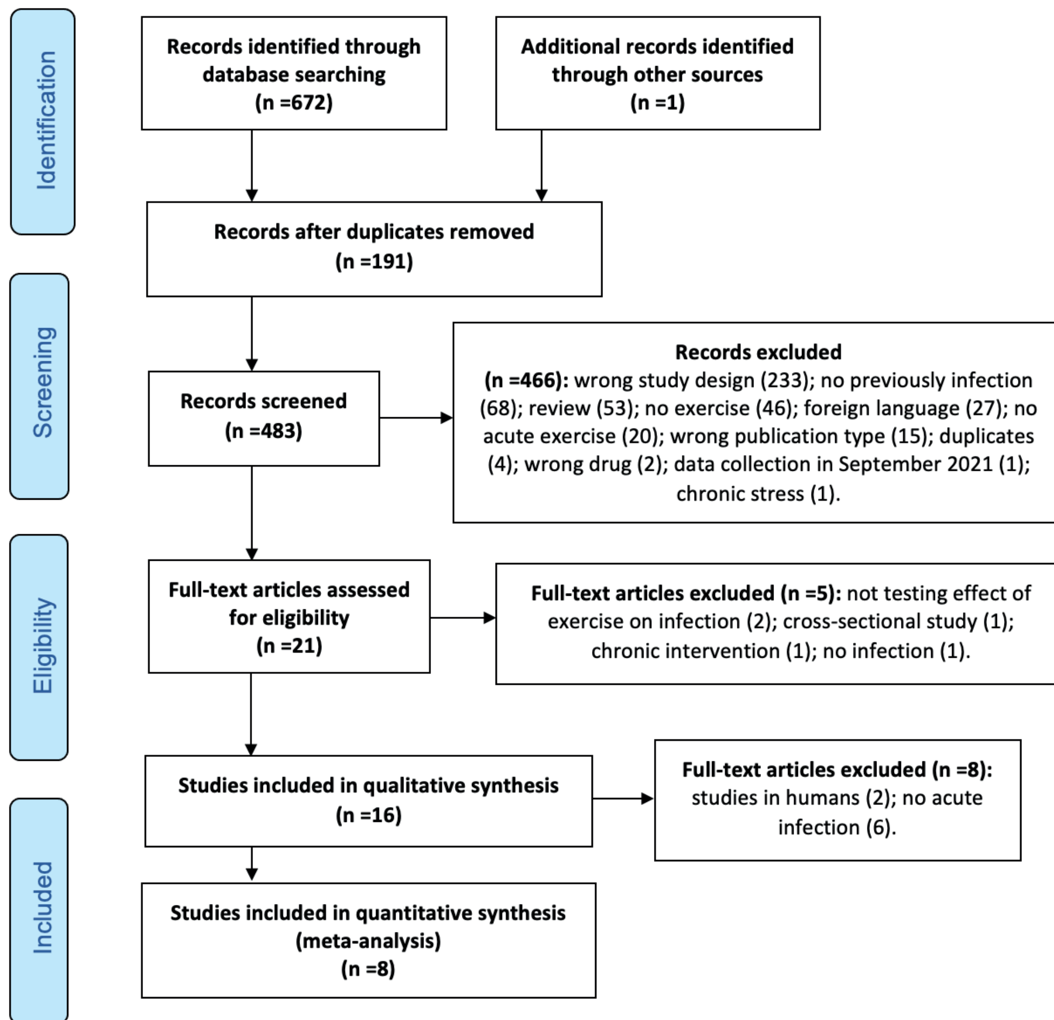
Supplementary Figure 1. The flowchart of selection of studies. Of note the final analysis did not include the 2 human studies and the focus was on the 8 animal studies.



Supplementary Table 1. SYRCLE Risk of Bias in the studies included.
Legend: 1: allocation sequence adequately generated and applied; 2: similar groups at baseline or adjusted for confounders in the analysis; 3: group allocation adequately concealed; 4: animals randomly housed during the experiment; 5: caregivers blinded; 6: animals selected at random for outcome assessment; 7:outcome assessor blinded; 8: incomplete outcome data adequately addressed; 9: Reports of the study free of selective outcome reporting; 10: apparently free of other risk of bias; NR: Not Reported.

First author, year	1	2	3	4	5	6	7	8	9	10	Total
Levinson, 1945	Yes	Yes	NR	Yes	No	NR	NR	NR	Yes	No	**4**
Davis,1997	Yes	Yes	NR	Yes	No	NR	NR	NR	Yes	No	**4**
Brown, 2004	Yes	Yes	NR	Yes	No	NR	NR	NR	Yes	No	**4**
Davis, 2004	Yes	Yes	NR	Yes	No	NR	NR	NR	Yes	No	**4**
Murphy, 2004	Yes	Yes	NR	Yes	No	NR	NR	NR	Yes	No	**4**
Lowder, 2005	Yes	Yes	NR	Yes	No	NR	NR	NR	Yes	No	**4**
Brown, 2007	Yes	Yes	NR	Yes	No	NR	NR	NR	Yes	No	**4**
Murphy, 2008	Yes	Yes	NR	Yes	No	NR	NR	Yes	Yes	No	**5**

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Department of Exercise Physiology and Sports Therapy
Institute of Sports Science, University of Giessen, Germany
Kugelberg 62 | 35394 Giessen
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