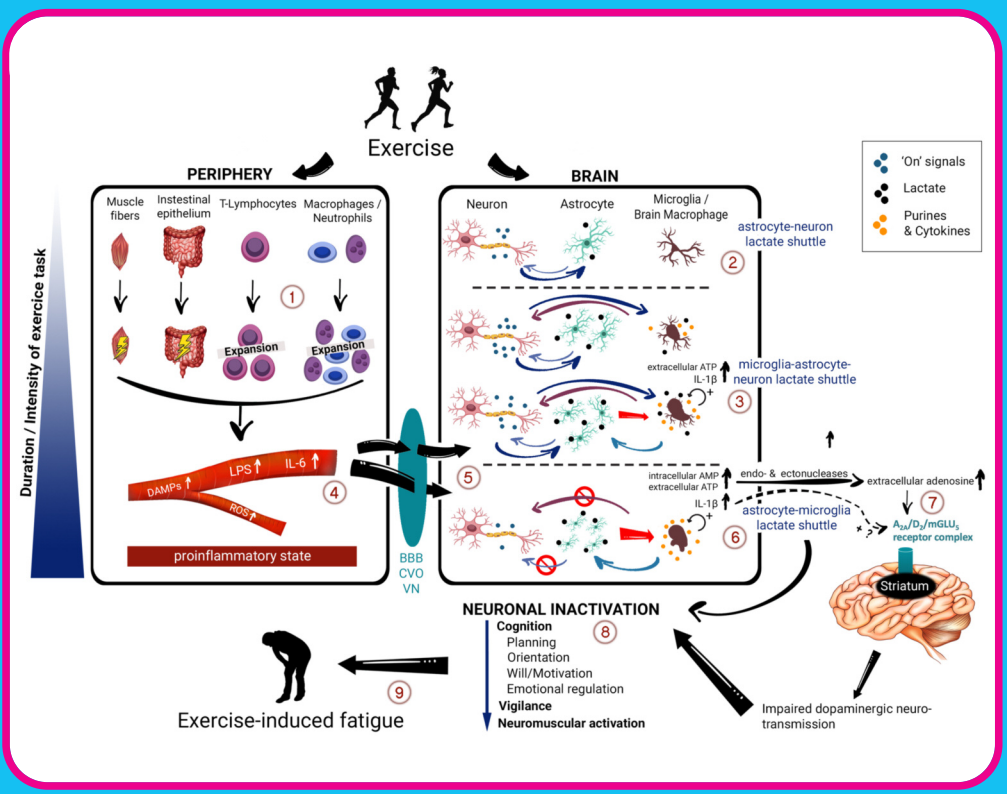
EXERCISE IMMUNOLOGY REVIEW



VOLUME 25 • 2019

The International Society of
Exercise and Immunology

EXERCISE IMMUNOLOGY REVIEW

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

EXERCISE IMMUNOLOGY REVIEW

VOLUME 25 • 2019

CONTENTS

Exercise Immunology Review

Editorial Statement

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

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

Exercise Immunology Review (ISSN 1077-5552)

Postmaster:
Prof. Dr. Karsten Krüger, Institute of Sport Science, Dept. of Exercise and Health, University of Hannover, Am Moritzwinkel 6, 30167 Hannover, Germany, karsten.krueger@sportwiss.uni-hannover.de

Editor-in-Chief: Prof. Dr. Karsten Krüger
Past Editor: Prof. Dr. Hinnak Northoff

Send editorial correspondence to:
Prof. Dr. Karsten Krüger
Institute of Sport Science, Dept. of Exercise and Health,
University of Hannover
Am Moritzwinkel 6,
30167 Hannover, Germany.
karsten.krueger@sportwiss.uni-hannover.de

Copyright © 2002 by Hinnak Northoff. *Exercise Immunology Review* is indexed in Sport Database, Sport Discus, Sport Search, SciSearch, EMBASE/ Excerpta Medica, Focus on: Sports Science & Medicine, Index Medicus, MEDLINE, Physical Education Index, Research Alert, International Bibliography of Periodical Literature, International Bibliography of Book Reviews, and CINAHL database.

Notice: authorization to photocopy items is granted for internal or personal use only. All other cases contact Karsten Krüger.

To subscribe or renew subscription go to http://www.isei.dk

From the Editors

EIR25 2019 contains seven articles. Fulvio Lauretani and colleagues were invited to contribute to EIR a highly relevant article about the role of exercise and the immune system as modulators of intestinal microbiome, including its implications for the gut-muscle axis hypothesis. The second article, written by Sebastian Proschinger and Jens Freese from the Sport University Cologne, represents a state-of-the-art article about neuroimmunological and neuroenergetic aspects in exercise-induced fatigue. Helena Batatinha (who incidentally received an honorary mention for ECR-award at the last ISEI meeting in Coimbra, Portugal) and colleagues contributed an article about specific inflammatory processes during obesity and smoke exposure, and analyzed the differences in the anti-inflammatory effects of exercise training for these two conditions. Glenn Wong and his team from Singapore present a comprehensive original study about the hallmarks of improved immunological responses to vaccination of physically active elderly females. The group of Ann Stowe present an interesting study about T and B cell subsets which differentially correlate with amyloid deposition and neurocognitive function in patients with amnestic mild cognitive impairment after one year of physical activity. A study by Michelle Curran and colleagues analyzed the impaired mobilization of highly-differentiated CD8+ T cells during a single bout of acute exercise in patients with type 1 diabetes. Finally, a murine study by Almeda-Viera and colleagues presents evidence that aerobic exercise reduces asthma phenotype through the involvement of SOCS-JAK-STAT signaling.

In 2018, a total of 21 manuscripts were submitted to EIR, which corresponds to an acceptance rate of about 30%. Officially, EIR has an impact factor of 7.105 (2017/18). For EIR26 and the future we would prefer, again, most contributions to be topical review articles. In the case of original research articles, we encourage the authors to embed their new data into review articles. Please note that the submission deadline for EIR26 is 31st July 2019. We hope you enjoy reading the new issue, and we hope to see you at the 14th ISEI symposium will be held in Shanghai, China, 8.-10th November 2019.

Thank you, Rickie Simpson, Neil Walsh and Jonathan Peake, for the continuing close, trusting and friendly teamwork. Thank you (all ISEI members), and all members of the Editorial Board for the confidence you have placed in us. Thank you all for your ongoing support of EIR. A special thanks to all the authors and reviewers of EIR25.

On behalf of the Editors,

Karsten Krüger

Exercise and immune system as modulators of intestinal microbiome: implications for the gut-muscle axis hypothesis

Andrea Ticinesi[1,2,3], Fulvio Lauretani[2*], Claudio Tana[2], Antonio Nouvenne[2,3], Erminia Ridolo[1,2], Tiziana Meschi[1,2,3]

[1] Department of Medicine and Surgery, University of Parma, Italy
[2] Geriatric-Rehabilitation Department, Azienda Ospedaliero-Universitaria di Parma, Italy
[3] Microbiome Research Hub, University of Parma, Italy

ABSTRACT

Exercise is a possible modulator of intestinal microbiome composition, since some investigations have shown that it is associated with increased biodiversity and representation of taxa with beneficial metabolic functions. Conversely, training to exhaustion can be associated with dysbiosis of the intestinal microbiome, promoting inflammation and negative metabolic consequences. Gut microbiota can, in turn, influence the pathophysiology of several distant organs, including the skeletal muscle. A gut-muscle axis may in fact regulate muscle protein deposition and muscle function. In older individuals, this axis may be involved in the pathogenesis of muscle wasting disorders through multiple mechanisms, involving transduction of pro-anabolic stimuli from dietary nutrients, modulation of inflammation and insulin sensitivity. The immune system plays a fundamental role in these processes, being influenced by microbiome composition and at the same time contributing to shape microbial communities. In this review, we summarize the most recent literature acquisitions in this field, disentangling the complex relationships between exercise, microbiome, immune system and skeletal muscle function and proposing an interpretative framework that will need verification in future studies.

Keywords: Gut microbiota; Sarcopenia; Inflammation; Exercise immunology; Sport

*Corresponding author:
Dr. Fulvio Lauretani, Department of Medicine and Surgery, University of Parma, Geriatric-Rehabilitation Department, Azienda Ospedaliero-Universitaria di Parma
Via Antonio Gramsci 14, 43126 Parma (Italy)
Tel: +39 3470912031, E-mail: flauretani@ao.pr.it

1. Introduction

1.1 The physiology of human intestinal microbiome

The human intestinal microbiome is composed of a complex ecosystem of more than 10^{14} bacteria, viruses, fungi, Protozoa and Archea that live symbiotically with the host in the gut lumen (45,61,82). Although there is increasing interest in the role of Protozoa, fungi (the "mycome") and viruses (the "virome"), most of the existing research has been focused mainly on bacteria, thanks to the availability of high-throughput sequencing techniques of bacterial DNA (16S rRNA microbial profiling, shotgun metagenomics) and fecal metabolomics (52,77,93,94).

The concentration of bacteria generally increases from the small intestine to the colon, and different bacterial populations are harbored in different tracts of the gastrointestinal system (123). Although recent research has shown that the fecal microbiota composition does not completely overlap with the intestinal mucosa-associated microbiota (134), gut microbiota composition has been determined from fecal samples in most studies (45,61,82,123).

It is generally agreed that the intestinal microbiome composition is shaped during early childhood, influenced by genetic and environmental factors. These factors include geography, delivery mode, breastfeeding, weaning, and exposure to environmental bacteria (34,54,78). The maturation of the gut microbiota towards the adult-type is reached by the age of 3 years (128). Interestingly, this process seems to exert a great influence on the development of the immune system, promoting immunogenic tolerance towards symbionts and immune activation against pathogens (40,50,110). Conversely, alterations in this process may induce a shift towards unappropriated type 2 immune responses, favoring the pathogenesis of allergy or autoimmune diseases, according to some theories (71,74).

In adulthood, the human gut microbiota composition remains relatively stable over time, exhibiting resilience to disruptors, such as stress, acute diseases, or antibiotic administration (65). This means that, after a brief exposure to disruptors, the microbiome faces a substantial, but transient, perturbation, followed by partial or total recovery of previous taxonomic composition (96).

The healthy microbiome includes a limited number of highly represented taxa, such as *Bacteroides* and *Prevotella* spp., and a large number (up to 2000 identified to date) of minor players with low representation but high metabolic activity (42,65). In

healthy subjects, microbiota composition shows a certain degree of inter-individual differences, that can be again explained by genetic (13) and environmental factors (28), such as the place of living, cultural habits, diet, diseases, medications, and immune system function (99). Dietary factors, and particularly carbohydrate and protein intake, are generally considered as the main determinants of gut microbiota composition (5,76,132).

In the older age, species richness and diversity of microbiome decline, inter-individual variability increases, and resilience to perturbations is reduced (24,86). Thus, the microbiome composition shifts towards a higher representation of opportunistic pathogens, and is related to increasing prevalence of malnutrition, frailty, disability and multimorbidity (49,112,114).

1.2 The intestinal microbiome in human diseases

A large body of literature has demonstrated that the presence of acute and chronic diseases, not limited only to the gastrointestinal system, is associated with alterations of the gut microbiota composition (67,99). These alterations, globally referred to as "dysbiosis", include reduced biodiversity, loss of commensals with possible beneficial metabolic activities and overgrowth of opportunistic pathogens (55,60,81). Dysbiosis implies a disruption of the mutual equilibrium between gut bacteria and host physiology (16). As a consequence, intestinal permeability increases, allowing bacteria or bacterial toxins and metabolites to enter into the host circulation and promote subclinical inflammation (64). Dysbiosis also reduces bioavailability of nutrients, affecting the microbial metabolism of several beneficial substances (73).

As a result, gut microbiota may exert a great influence on the functionality and pathophysiology of several organs anatomically distant from the gastrointestinal tract. For example, gut microbiota dysbiosis may be involved in the pathophysiology of dementia (17,115), Parkinson's disease (108) ("gut-brain axis"), chronic kidney disease (95), nephrolithiasis (113) ("gut-kidney axis"), asthma (73) ("gut-lung axis") and even osteoporosis (84) ("gut-bone axis").

However, most of these associations linking dysbiosis with extra-intestinal diseases have been demonstrated in studies with a cross-sectional design. Longitudinal studies, demonstrating a causal relationship between microbiota composition and disease onset and course, are still lacking (99). Thus, the real clinical implications of gut microbiota dysbiosis and interventions targeted at modifying gut microbiota composition are still poorly understood.

2. Exercise and gut microbiota: the yin and yang

2.1 Beneficial effects of exercise on gut microbiota

Exercise is considered as one of the main environmental factors possibly influencing gut microbiota composition (99). The complex relationship between exercise and microbiome, and its possible implications for athletic performance have already been reviewed elsewhere (10,27,87). However, research in this field has made substantial improvements in the last few years, and some recent acquisitions deserve to be mentioned and discussed.

Exercise is generally considered a positive modulator of gut microbiota biodiversity. This concept has been supported by investigations performed in animals (8,18,57,75), and then confirmed in human studies.

In a case-control study, microbial diversity was much higher in a group of professional rugby players than in age-, sex- and body size-matched controls not performing sports (26). Recently, shotgun metagenomics analyses of the fecal samples from the same groups highlighted that athletes had a different microbiome composition also from a functional point of view, with increased microbial representation of genes involved in carbohydrate and amino acid metabolism, and short-chain fatty acid (SCFA) production (7). In another study, the average abundance of taxa involved in energy and carbohydrate metabolism, including *Prevotella* and *Methanobrevibacter smithii,* resulted significantly higher in professional than amateur cyclists, and was correlated with the frequency of training (89). However, these studies could not fully disentangle the contribution of exercise and diet in determining different microbiota compositions in different groups, since participants followed a wide range of dietary regimens.

The intensity of training is also important: light exercise programs induce only subtle modifications of gut microbiota composition in sedentary subjects (31). Therefore, the findings of studies performed in athletes should not automatically be transferred to all subjects undertaking non-competitive exercise.

According to three different studies (37,38,127), fecal microbiota biodiversity is correlated with cardiorespiratory fitness in adult subjects. However, in one of these studies, performed in 71 premenopausal Finnish women, this relationship was mediated by body composition (127). Another study, performed in 19 active and 21 sedentary women aged ≤40 years old, confirmed that the microbiome abundance of several bacterial taxa was significantly correlated with the body fat or lean mass percentage (15). Thus, the possible association between exercise and microbiota should be further investigated, carefully taking into account possible confounders, such as dietary habits, nutrient intake, and parameters of body composition.

The influence of body composition on microbiota was emphasized also by the findings of one intervention study, where two groups of sedentary subjects, one lean and one obese, underwent a 6-week structured exercise program, followed by a 6-week washout period (2). After exercise training, both lean and obese participants experienced a change in gut microbiota composition, but the overall representation of species with known anti-inflammatory properties and the microbiome capacity of producing SCFA was higher in lean subjects, highlighting a body mass index (BMI)-dependent response to training. However, all the changes reversed towards the baseline status after the washout period (2). Interestingly, in exercised healthy young males undergoing a period of forced inactivity, cessation of exercise was associated with changes in gastrointestinal physiology (i.e. reduction of bowel movements and increased consistency of feces) before alterations of gut microbiota composition and function could be detected (103,104,105). These circumstances suggest that the microbiome is resilient to acute changes in exercise habits, and that maintenance of exercise is needed to induce long-lasting modifications of intestinal microbial ecosystem.

The modifications of intestinal microbiome composition induced by exercise can exert beneficial effects on the whole organism, modulating pathological processes. For example, exercise-induced microbiota changes are able to attenuate the

clinical course and outcome of experimental models of myocardial infarction or chemically-induced colitis, especially by modulating the inflammatory response (1,63). The key mediators in these processes may be SCFA, and particularly butyrate, whose production by gut microbiota has been shown to increase after exercise in humans (2).

2.2 Negative effects of exercise on gut microbiota

Despite the findings of the studies discussed above, other investigations have questioned the concept that the exercise-induced changes in gut microbiota composition are always favorable for the host physiology. Endurance high-intensity exercise, especially if not proportioned to training level, may in fact represent a huge stressor for the organism. These conditions can induce ischemic events in the gut mucosa, associated with acute gastrointestinal symptoms including abdominal pain, nausea, and diarrhea (32). From a gut microbiota perspective, these phenomena may be associated with increased intestinal permeability allowing several bacteria and their toxic products to enter systemic circulation and activate systemic inflammation (51,97). A basic mediator in these processes is represented by microbiota-derived lipopolysaccharide (LPS) (97), exerting a wide range of pathological actions on the host (4).

Moreover, the high-intensity exercise-induced dysfunction of the intestinal mucosa may promote profound and rapid changes in microbiota. For example, in a group of soldiers, a 4-day military training program of Arctic cross-country ski-march resulted in deep changes in fecal microbiota composition and functionality. Namely, there was an expansion of a large number of taxa, including opportunistic pathogens, at the expense of dominant taxa, such as *Bacteroides,* and taxa with known production of anti-inflammatory mediators (51). In amateur athletes, the fecal microbiome functionality acutely changed after a half-marathon race, exhibiting a pro-inflammatory profile with a completely different fecal metabolome (131). Similar changes have also been demonstrated in animal models (129). Interestingly, the administration of probiotics or prebiotics seems to attenuate these unfavorable changes of gut microbiota after exercise to exhaustion (22,48,97), although the benefits are uncertain in case of lower intensity of exercise (118,119).

In summary, regular exercise training seems to be associated with higher biodiversity and beneficial functions of intestinal microbiome. The microbiota may thus represent a mediator of the exercise-induced health benefits, although diet and body composition may play a relevant role in this association. On the other side, there are also some studies supporting that exercise to exhaustion may be associated with detrimental consequences for the microbiome. The effects of exercise on the intestinal microbiome may thus depend on its intensity and timing, and future studies should help to disentangle this relationship.

3. Can the microbiota influence muscle pathophysiology? The gut-muscle axis hypothesis in age-related sarcopenia

Recently, several research groups have independently hypothesized that the gut microbiota composition may influence the emergence of sarcopenia, i.e. the loss of muscle mass and function occurring with aging (33,44,83,90,112,116). A study performed on rat models of sarcopenia has actually demonstrated that age-related muscle mass wasting is associated with a distinct fecal microbiota composition, with reduced representation of several taxa with purported anti-inflammatory and pro-anabolic actions on the host tissues, including *Clostridium* XIVa cluster, *Butyricicoccus, Sutterella, Coprococcus* and *Faecalibacterium* (102). Sarcopenic rats also exhibited a different fecal microbiota functionality, with rearrangements in the expression of bacterial genes involved in nutrient biosynthesis and catabolism (102).

To date, no studies have investigated the composition and functionality of fecal microbiota in older humans with sar-

Table 1: Overview of the possible pathophysiological mechanisms involved in the gut microbiota-mediated regulation of skeletal muscle function (gut-muscle axis) and of the corresponding effects in case of gut microbiota dysbiosis.

Hypothesized pathways involved in gut microbiota modulation of muscle function	Possible skeletal muscle effects in case of dysbiosis
Bioavailability of dietary proteins and specific amino acids (tryptophan) (36,62,101)	↓ protein synthesis ↓ IGF-1-mediated anabolic effect
Synthesis of vitamins, including folate, vitamin B12, riboflavin (59,111)	↓DNA synthesis, methylation and repair ↓oxidative stress neutralization capacity ↓amino acid biosynthesis ↓energy production
Biotransformation of nutrients, including polyphenols (resveratrol) and ellagitannins (97,106)	↓mitochondrial biogenesis ↓ exercise resistance capacity ↓ oxidative stress neutralization capacity
Permeability of intestinal mucosa (20,25)	↑ inflammation (stimulation of TLRs) ↑ muscle protein catabolism ↓ insulin sensitivity with anabolic resistance
Biotransformation of bile acids (53)	↓ activation of anabolic pathways regulated by farnesoid X receptor
Synthesis of short-chain fatty-acids (19,118,123)	↓ stimulation of anabolism ↓ insulin sensitivity ↑ systemic and local pro-inflammatory cytokine production

IGF-1 = Insulin-Like Growth-Factor-1; TLR = Toll-Like Receptors

copenia (116). However, there is some indirect evidence supporting the hypothesis of a gut-muscle axis, in which the intestinal microbiota composition can influence muscle mass anabolism and functionality.

The physio-pathological substrates of sarcopenia are represented by reduced muscle capillarity, reduced insulin sensitivity, and increased subclinical inflammation, resulting in altered mitochondrial biogenesis and function, and altered anabolic/catabolic balance of muscle protein synthesis (14,69,70). From a clinical point of view, muscle mass loss may also be favored by several conditions that are frequently found in geriatric patients, including malnutrition, low dietary protein intake, intestinal malabsorption, altered digestion and subclinical cognitive deficits (58,83).

In this context, the intestinal microbiota composition may influence the onset of sarcopenia at multiple levels. The presence of gut microbiota dysbiosis is in fact associated with several metabolic alterations, involving protein synthesis, release of pro-anabolic mediators, inflammation and insulin sensitivity. All these elements can modulate skeletal muscle physiology, as summarized in Table 1.

First, a dysbiotic intestinal microbiota can reduce the bioavailability of dietary proteins (102) and particularly of some amino acids, like tryptophan, involved in modulation of inflammation and promotion of muscle protein synthesis (21,36,62). Gut bacteria are also involved in the synthesis of many vitamins, including folate, vitamin B12 and riboflavin, exerting several beneficial and pro-anabolic effects in skeletal muscle cells, ranging from amino acid biosynthesis to oxidative stress neutralization during exercise (59).

Moreover, a healthy intestinal microbiota can effectively transform some dietary nutrients into metabolic mediators that, once absorbed into systemic circulation, can exert beneficial effects on inflammation, insulin sensitivity, anabolism, and antioxidant capacity. Conversely, a dysbiotic microbiota may lack these functions, with some negative consequences on muscle health. Polyphenols, including resveratrol, and ellagitannins contained in pomegranates and berries represent the most relevant examples of nutrients that, after microbial metabolism, enter systemic circulation and exert beneficial effect for the muscle (98,107). Interestingly, endurance training seems to enhance the bioavailability of dietary polyphenols, probably through its beneficial modulations of intestinal microbiota (88).

Moreover, the age-related alterations of gut microbiota composition (24), occurring independently from the level of exercise training, can promote gut mucosa dysfunction, with increased permeability. This phenomenon may result in the systemic absorption of microbial byproducts and toxins, including LPS (20). In skeletal muscle cells, circulating LPS can contribute to activate Toll-Like Receptors (TLR) 4 and 5, promoting NF-kB pathway activation, with reduced insulin sensitivity, enhanced protein catabolism and inflammatory cytokine production (72,106). In animal models, TLR4 activation determines muscle atrophy (35). In aging human beings, TLR4 activation is associated with metabolic endotoxinemia, decreased insulin sensitivity and reduced quadriceps muscle strength and volume (41).

But probably the most studied mechanism involved in gut microbiota modulation of muscle function is the bacterial production of metabolic mediators, including bile acids and SCFA (20,25). A healthy gut microbiota can produce secondary bile acids, that are well known activators of farnesoid X receptor stimulating myocyte anabolism (53). SCFA, and particularly butyrate, are generally synthetized by a large number of gut bacteria, including *Faecalibacterium, Butyricimonas,* and *Succinivibrio*, highly represented in healthy subjects but with reduced abundance in older individuals (19). These mediators have several beneficial metabolic activities, summarized in Table 2, ultimately influencing skeletal muscle protein deposition through modulation of the systemic anabolic/catabolic balance (6,19). The administration of butyrate and probiotics with similar functionality to animal models of muscle wasting resulted in massive improvements in muscle mass (120,125). Unfortunately, human studies on this topic are still lacking to date.

The metabolic action of gut microbiota was however confirmed in a study by Blanton and colleagues, where the transplantation of the dysbiotic fecal microbiota from malnourished African children to germ-free mice resulted in mouse failure-to-thrive (12).

Furthermore, the administration of rifaximin to mouse models of hepatic encephalopathy surprisingly resulted in improved skeletal muscle mass and function (56). Rifaximin is able to

Table 2: Summary of the main physiological functions of short-chain fatty acids (butyrate, acetate, propionate) produced by the intestinal microbiota (6,19,118,123). The most relevant functions possibly involved in the gut-muscle axis are shown in italics.

Substance	Function
Acetate	*Direct modulation of systemic inflammation*
	Antagonization of LPS-driven inflammation
	Improvement in insulin sensitivity
	Stimulation of skeletal muscle glucose uptake
	Peripheral modulation of satiety via GLP-1 and PYY
	Central appetite modulation
	Promotion of lipolysis
	Induction of adipose tissue differentiation
	Increased energy expenditure via thermogenesis
Propionate	*Increased Treg cell differentiation*
	Direct modulation of systemic inflammation
	Antagonization of LPS-driven inflammation
	Peripheral modulation of satiety via GLP-1 and PYY
	Promotion of lipolysis
	Induction of adipose tissue differentiation
	Increased energy expenditure via thermogenesis
Butyrate	*Direct modulation of systemic inflammation*
	Antagonization of LPS-driven inflammation
	Improvement in insulin sensitivity
	Stimulation of skeletal muscle glucose uptake
	Histone deacetylase inhibition
	Reduced intestinal permeability
	Peripheral modulation of satiety via GLP-1 and PYY
	Central appetite modulation
	Promotion of lipolysis
	Induction of adipose tissue differentiation
	Increased energy expenditure via thermogenesis

GLP-1 = Glucagon-Like Peptide-1; PYY = Peptide YY; LPS = Lipopolysaccharide

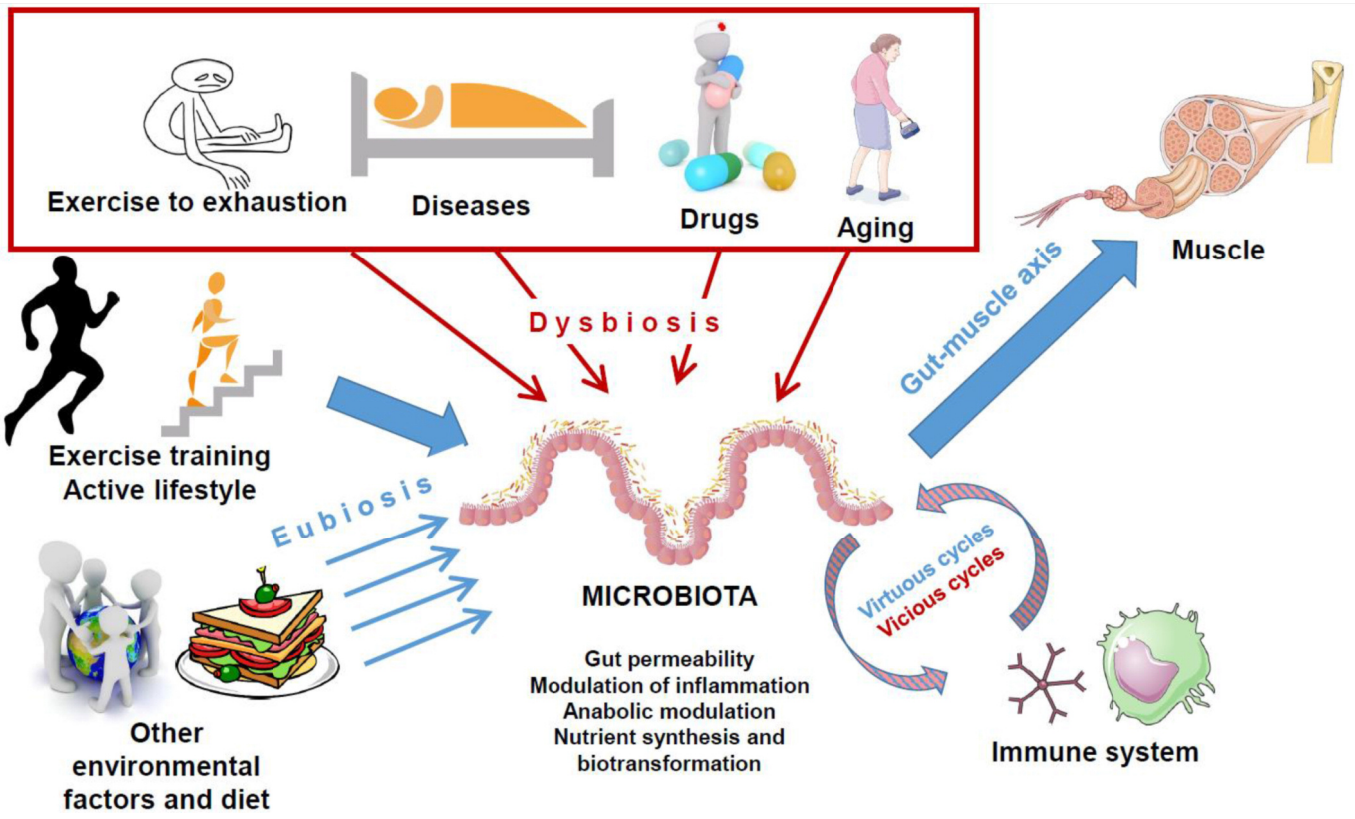


Figure 1: Representation of the hypothetical conceptual framework connecting exercise, intestinal microbiome, inflammation, immune system function and skeletal muscle pathophysiology at the current literature state of art.

selectively kill pathobionts, favoring the expansion of bacterial populations with purported beneficial activities, such as Bifidobacteria or Lactobacilli (91). The observed effects on mice may depend on its capacity to reduce the gut microbiota dysbiosis associated with hepatic encephalopathy.

In summary, the results of several pre-clinical studies support the hypothesis that gut microbiota dysbiosis may be associated with muscle wasting, especially in age-related sarcopenia. However, confirmation of this possible gut-muscle axis in human studies is still lacking, and the clinical relevance of these supposed mechanisms is still uncertain.

4. Exercise and the gut-muscle axis

Although studies on humans are lacking, several preclinical studies support the hypothesis that the intestinal microbiota can modulate skeletal muscle physiology not only in age-related sarcopenia, but in all ages and physiological states (20,25). In this context, exercise may represent a strong modulator of gut microbiota composition. Thus, the gut-muscle communication in human pathophysiology may be bidirectional (25), with gut microbiota representing a "cross-road" among environment, lifestyle, and skeletal muscle (112).

In this scenario, some authors have hypothesized that many of the well-known positive health effects of exercise may be mediated by its beneficial modifications on the gut microbiota (23,79,80). However, when there is an exercise overload, these possible beneficial effects are overweighed by increased intestinal permeability and oxidative stress, promoting inflammation and a catabolic state that negatively impacts the functionality of skeletal muscle (29). Moreover, the harmful effects of inactivity on the muscle and vascular system may be at least partly mediated by negative changes of the gut microbiota towards dysbiosis (23,109).

In healthy subjects who regularly perform physical activity, a homeostatic equilibrium between intestinal microbiota and skeletal muscle may be present, with exercise promoting healthy microbiota composition, and microbiota favoring muscle health. This equilibrium may be disrupted by sedentary lifestyle or excessive exercise, resulting in dysbiosis of the gut microbiota. Other factors promoting dysbiosis, such as drugs or acute illnesses, may also be associated with reduced muscle mass and function. In fact, dysbiosis influences gut permeability, systemic inflammation, anabolism and nutrient availability. All these mechanisms are involved in muscle physiology and represent the substrates of the gut-muscle axis, as depicted in Figure 1.

In summary, the gut-muscle axis may be two-way, with microbiota influencing the muscle, and exercise contributing to shape microbiota composition. The intensity and frequency of exercise may have great importance in determining which way of the axis is prevalent, and its physio-pathological consequences.

5. Immune system and the gut-muscle axis: virtuous and vicious cycles

A healthy gut microbiota has a fundamental role in shaping local and systemic immune response to gut bacteria through the whole lifespan, favoring the maintenance of tolerance towards antigens from commensals and activation against antigens from pathogens (40). On the other side, gut micro-

biota dysbiosis favors the loss of immunologic tolerance to commensals, the impairment of epithelial barrier function and an inbalance in the activation of anti-inflammatory T_{reg} lymphocytes and pro-inflammatory Th17 lymphocytes (43,60). These phenomena may contribute to the onset of several infectious, inflammatory and autoimmune diseases, including inflammatory bowel diseases, type 1 diabetes and multiple sclerosis, with gut microbiota playing an active pathogenic role (9).

However, besides this "outside-in" relationship, there is also an important "inside-out" control of immune system over gut microbiota (47,117). The immune system is in fact able to influence the gut microbiota composition at multiple levels. Both innate and adaptive immunity are involved. The possible mechanisms are synthetized in Table 3, and include production of antimicrobial peptides from intestinal cells, mucus secretion, immunoglobulin A (IgA) activation, toll-like receptor (TLR) activation, lymphocyte transfer and differentiation, presence of invariant natural killer T cells (iNKTC) (47,60,68,101,130). The presence of specific inbalances in each one of these pathways may be associated with the emergence of gut microbiota dysbiosis (47,68).

In fact, human beings infected with the Human Immunodeficiency Virus (HIV) exhibit deep changes in the structure and functionality of the intestinal microbiome, with increased biodiversity due to overgrowth of opportunistic pathogens and decreased representation of taxa with anti-inflammatory properties (121,122,133). Similar alterations of gut microbial community structure have been detected also in patients with IgA deficiency (39). These findings support the concept that the immune system functionality influences gut microbiota composition.

Conversely, the presence of specific functionalities in the gut microbiome, related to fatty acid metabolism, PPAR-signaling, lipid biosynthesis and kynurenine pathway of tryptophan metabolism, may enhance systemic immunity activation and promote control of HIV infection (124). Therefore, a complex interplay between microbiome and immunity exists, and the physio-pathological consequences depend on the type of equilibrium reached (117).

Some metabolic mediators, such as bile salts, may play a relevant role in this equilibrium. In the gut lumen, bile salts can in fact undergo metabolic transformations into compounds with immunoregulatory and anti-inflammatory properties, particularly on Kupffer cells and intrahepatic lymphocytes (100). Bile salts have also the capacity of selecting specific subpopulations of the gut microbiota that are able to metabolize them, contributing to shape the intestinal microenvironment (100).

Table 3: Overview of the mechanisms involved in immune system control of gut microbiota composition (47,60,68,100,128).

Immunity type	Mechanism
Intestinal innate immunity	Production of mucus (barrier function)
	Production of antimicrobial peptides by Paneth cells
	Production of α-defensins by epithelial cells
	Activation of NOD-like receptor and production of IL-18 by epithelial cells
	Release of non-specific immunoglobulins
	Invariant Natural Killer lymphocyte activation
	Expression of Resistin-like molecule β
	Leptin expression
Intestinal acquired immunity	IgA response to gut microbiota antigens
	Activation of CD4+ T cells in intestinal mucosa
	Activation of Foxp3+ T cells in intestinal mucosa
	Activation of Toll-Like Receptors with lymphocyte stimulation
	HLA class I and II loci expression
Systemic acquired immunity	IgG response to gut microbiota antigens penetrated in systemic circulation

Table 4: Summary of the main features of aging immune system involved in increased gut mucosa permeability and in age-related gut microbiota dysbiosis (11,66,124).

Immunity type	Age-related alterations
Innate	Reduced Paneth cell function
	Reduced glycosylation of mucins
	Reduction of M cell number and function
	Increased levels of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α
	Decreased levels of anti-inflammatory cytokines including IL-10
	Increased production of adipokines
	Impaired phagocytic function of macrophages
	Overgrowth of pro-inflammatory $CX3CR1^{Int}$ macrophages
	Reduced chemotactic capacity in neutrophils
Acquired	Reduced antigen presentation capacity by dendritic cells
	Reduced T cell priming by dendritic cells
	Reduced differentiation of CD4+ CD25+ T cells
	Reduced T cell secretion of TGF-β
	Increased T cell secretion of IFN-γ
	Overgrowth of T_{reg} lymphocytes
	Reduced homing of IgA-secreting B lymphocytes
	Reduced IgA secretion capacity
	Loss of naïve T and B cells

Whatever the mediators involved, the equilibrium between immune system and microbiome may be strongly influenced by environmental factors. Positive modulators of gut microbiota composition, including regular exercise, may induce a beneficial equilibrium with the immune system, resulting in a virtuous cycle helping to maintain health (27,85). Conversely, factors that disrupt gut microbiota composition, such as exercise to exhaustion, illness and aging, may cause a perturbation of the equilibrium between microbiome and immune system. As a result, systemic inflammation is chronically activated, sustaining further alterations of the microbiota towards dysbiosis promoted by the altered immune system regulation (30,46). So, a vicious circle arises.

These postulated mechanisms are highlighted in Figure 1.
A healthy gut microbiota, and a positive interaction with the immune system, may be crucial for the gut-muscle axis, and may influence the maintenance of muscle mass and functionality, especially in exercised subjects (29). Conversely, dysbiosis resulting from a negative interaction with the immune system may influence muscle wasting disorders, particularly during aging (112).
Age-related gut microbiota dysbiosis is associated with increased gut mucosa permeability in both animal models and humans (92,111). The reduced intestinal epithelial barrier function is accompanied by several alterations in immune system, involving both innate and acquired immunity (Table 4) (11,66,126). These alterations ultimately promote local and systemic inflammation, with overproduction of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 (66,126). Inflammation negatively impacts the gut-muscle axis and is involved in the pathogenesis of several age-related conditions, including not only sarcopenia and frailty (126), but even cancer (11). Moreover, IL-1β further stimulates intestinal epithelial tight junction permeability and promotes local dysbiosis (3), in a vicious cycle supporting skeletal muscle wasting and loss of function.
In summary, the relationship between intestinal microbiome and immunity may be two-way, and the resulting equilibrium may exert important functions on the functionality of the gut-muscle axis and on muscle health. More research is however needed to disentangle these complex relationships, and to reveal their actual relevance from a clinical perspective.

6. Conclusions

The relationship between exercise, immune system, gut microbiota, and skeletal muscle pathophysiology is very complex and not completely elucidated at the current state of the art. In Figure 1, we present a possible interpretative framework, showing that the gut microbiota is at the cross-road between environmental stimuli and host physiology, undergoing a continuous interplay with the immune system and the skeletal muscle.
Future studies should clarify whether gut microbiota dysbiosis is pathophysiologically associated with muscle wasting disorders, and if exercise may positively influence this putative gut-muscle axis. Furthermore, the influence of the microbiome-immune system interplay on skeletal muscle mass and functionality should be investigated in both experimental models and human beings.

ACKNOWLEDGMENTS

Part of the images are distributed under Creative Commons Licence and can be freely available at the following links: https://smart.servier.com/ and https://pixabay.com.
The authors wish to thank Dr. Cristoforo Incorvaia for advice on manuscript writing.

FINANCIAL SUPPORT AND SPONSORSHIP

None.

COMPETING INTERESTS

All the authors have no conflict of interest to declare.

REFERENCES

1. Allen JM, Mailing LJ, Cohrs J, Salmonson C, Fryer JD, Nehra V, Hale VL, Kashyap P, White BA, Woods JA. Exercise training-induced modification of the gut microbiota persists after microbiota colonization and attenuates the response to chemically-induced colitis in gnotobiotic mice. Gut Microbes 9: 115-130, 2018.
2. Allen JM, Mailing LJ, Niemiro GM, Moore R, Cook MD, White BA, Holscher HD, Woods JA. Exercise alters gut microbiota composition and function in lean and obese humans. Med Sci Sports Exerc 50: 747-757, 2018.
3. Al-Sadi R, Ye D, Said HM, Ma TY. IL-1β-induced increase in intestinal epithelial tight junction permeability is mediated by MEKK-1 activation of canonical NF-κB pathway. Am J Pathol 177: 2310-2322, 2010.
4. Armstrong LE, Lee EC, Armstrong EM. Interactions of gut microbiota, endotoxinemia, immune function, and diet in exertional heatstroke. J Sports Med 2018: 5724575, 2018.
5. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, Bertalan M, Borruel N, Casellas F, Fernandez L, Gautier L, Hansen T, Hattori M, hayashi T, Kleerebezem M, Kurokawa K, Leclerc M, Levenez F, Manichanh C, Nielsen HB, Nielsen T, Pons N, Poulain J, Qin J, Sicheritz-Ponten T, Tims S, Torrents D, Ugarte E, Zoetendal EG, Wang J, Guarner F, Pedersen O, de Vos WM, Vrunak S, Doré J, MetaHIT Consortium. Enterotypes of the human gut microbiome. Nature 473: 174-180, 2011.
6. Backhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci USA 104: 979-984, 2007.
7. Barton W, Penney NC, Cronin O, Garcia-Perez I, Molloy MG, Holmes E, Shanahan F, Cotter PD, O'Sullivan O. The microbiome of professional athletes differs from that of more sedentary subjects in composition and particularly at the functional metabolic level. Gut 67: 625-633, 2018.
8. Batacan RB, Fenning AS, Dalbo VJ, Scanlan AT, Duncan MJ, Moore RJ, Stanley D. A gut reaction: the combined influence of exercise and diet on gastrointestinal microbiota in rats. J Appl Microbiol 122: 1627-1638, 2017.
9. Belkaid Y, Hand TW. Role of microbiota in immunity and inflammation. Cell 157: 121-141, 2014.
10. Bermon S, Petriz B, Kajeniene A, Prestes J, Castell L, Franco OL. The microbiota: an exercise immunology perspective. Exerc Immunol Rev 21: 70-79, 2015.
11. Biragyn A, Ferrucci L. Gut dysbiosis: a potential link between increased cancer risk in ageing and inflammaging. Lancet Oncol 19: e295-e304, 2018.
12. Blanton LV, Charbonneau MR, Salih T, Barratt MJ, Venkatesh S, Ilkaveya O, Subramanian S, Manary MJ, Trehan I, Jorgensen JM, Fan YM, Henrissat B, Leyn SA, Rodionov DA, Osterman AL, Maleta KM, Newgard CB, Ashorn P, Dewey KG, Gordon JI. Gut bacteria that prevent growth impairments transmitted by microbiota from malnourished children. Science 351: aad3311, 2016.

13. Bonder MJ, Kurilshikov A, Tigchelaar EF, Mujagic Z, Imhann F, Vila AV, Deelen P, Vatanen T, Schirmer M, Smeekens SP, Zhernakova DV, Jankipersadsing SA, Jaeger M, Oosting M, Cenit MC, Masclee AA, Swertz MA, Li Y, Kumar V, Joosten L, Harmsen H, Weersma RK, Franke L, Hofker MH, Xavier RJ, Jonkers D, Netea MG, Wijmenga C, Fu J, Zhernakova A. The effect of host genetics on the gut microbiome. Nat Genet 48: 1407-1412, 2016.
14. Bowen TS, Schuler G, Adams V. Skeletal muscle wasting in cachexia and sarcopenia: molecular pathophysiology and impact of exercise training. J Cachexia Sarcopenia Muscle 6: 197-207, 2015.
15. Bressa C, Bailen-Andrino M, Perez-Santiago J, Gonzalez-Soltero R, Perez M, Montalvo-Lominchar MG, Maté-Munoz JL, Dominguez R, Moreno D, Larrosa M. Differences in gut microbiota profile between women with active lifestyle and sedentary women. PLoS One 12: e0171352, 2017.
16. Buttò LF, Schauebeck M, Haller D. Mechanisms of microbe-host interaction in Crohn's disease: dysbiosis vs pathobiont selection. Front Immunol 6: 555, 2015.
17. Calvani R, Picca A, Lo Monaco MR, Landi F, Bernabei R, Marzetti E. Of microbes and minds: an narrative review on the second brain aging. Front Med 5: 53, 2018.
18. Campbell SC, Wisniewski PJ, Noji M, McGuinness LR, Haggblom MM, Lightfoot SA, Joseph LB, Kerkhof LJ. The effect of diet and exercise on intestinal integrity and microbial diversity in mice. PLoS One 11: e0150502, 2016.
19. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nat Rev Endocrinol 11: 577-591, 2015.
20. Cerdà B, Perez M, Perez-Santiago JD, Tornero-Aguilera JF, Gonzalez-Soltero R, Larrosa M. Gut microbiota modification: another piece in the puzzle of the benefits of physical exercise in health? Front Physiol 7: 51, 2016.
21. Cervenka I, Agudelo LZ, Ruas JL. Kynurenines: tryptophan's metabolites in exercise, inflammation, and mental health. Science 357: 369; 2017.
22. Chaves FM, Baptista IL, Simabuco FM, Quaresma PGF, Pena FL, Bezerra RMN, Pauli JR, da Cunha DT, Campos-Ferraz PL, Antunes AEC. High-intensity-exercise-induced intestinal damage is protected by fermented milk supplemented with whey protein, probiotic and pomegranate (Punica granatum L.). Br J Nutr 119: 896-909, 2018.
23. Chen J, Guo Y, Gui Y, Xu D. Physical exercise, gut, gut microbiota, and atherosclerotic cardiovascular diseases. Lipids Health Dis 17: 17, 2018.
24. Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HM, Coakley M, Lakshminarayanan B, O'Sullivan O, Fitzgerald GF, Deane J, O'Connor M, Harnedy N, O'Connor K, O'Mahony D, van Sinderen D, Wallace M, Brennan L, Stanton C, Marchesi JR, Fitzgerald AP, Shanahan F, Hill C, Ross RP, O'Toole PW. Gut microbiota composition correlates with diet and health in the elderly. Nature 488: 178-184, 2012.
25. Clark A, Mach N. The crosstalk between the gut microbiota and mitochondria during exercise. Front Physiol 8: 319, 2017.
26. Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, Hogan A, Hayes P, O'Reilly M, Jeffery IB, Wood-Martin R, Kerins DM, Quigley E, Ross RP, O'Toole PW, Molloy MG, Falvey E, Shanahan F, Cotter PD. Exercise and associated dietary extremes impact on gut microbial diversity. Gut 63: 1913-1920, 2014.
27. Codella R, Luzi L, Terruzzi I. Exercise has the guts: how physical activity may positively modulate gut microbiota in chronic and immune-based diseases. Dig Liver Dis 50: 331-341, 2018.
28. Conlon MA, Bird AR. The impact of diet and lifestyle on gut microbiota and human health. Nutrients 7: 17-44, 2014.
29. Cook MD, Allen JM, Pence BD, Wallig MA, gaskins HR, White BA, Woods JA. Exercise and gut immune function: evidence of alterations in colon immune cell homeostasis and microbiome characteristics with exercise training. Immunol Cell Biol 94: 158-163, 2016.
30. Cook MD, Martin SA, Williams C, Whitlock K, Wallig MA, Pence BD, Woods JA. Forced treadmill exercise training exacerbates inflammation and causes mortality while voluntary wheel training is protective in a mouse model of colitis. Brain Behav Immun 33: 46-56, 2013.
31. Cronin O, Barton W, Skuse P, Penney NC, Garcia-Perez I, Murphy EF, Woods T, Nugent H, Fanning A, Melgar S, Falvey EC, Holmes E. Cotter PD, O'Sullivan O, Molloy MG, Shanahan F. A prospective metagenomics and metabolomics analysis of the impact of exercise and/or whey protein supplementation on the gut microbiome of sedentary adults. mSystems 3: e00044-18, 2018.
32. de Oliveira EP, Burini R. The impact of physical exercise on the gastrointestinal tract. Curr Opin Clin Nutr Metab Care 12: 533-538, 2009.
33. de Sire R, Rizzatti G, Ingravalle F, Pizzoferrato M, Petito V, Lopetuso L, Graziani C, de Sire A, Mentella MC, Mele MC, Gasbarrini A, Scaldaferri F. Skeletal muscle-gut axis: emerging mechanisms of sarcopenia for intestinal and extra-intestinal diseases. Minerva Gastroenterol Dietol 64: 351-362, 2018.
34. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, Knight R. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci USA 107; 11971-11975, 2010.
35. Doyle A, Zhang G, Abdel Fattah EA, Eissa NT, Li YP. Toll-like receptor 4 mediates lipopolysaccharide-induced muscle catabolism via coordinate activation of ubiquitin-proteasome and autophagy-lysosome pathways. FASEB J 25: 99-110, 2011.
36. Dukes A, Davis C, El Refaey M, Upadhyay S, Mork S, Arounleut P, Johnson MH, Hill WD, Isales CM, Hamrick MW. The aromatic amino acid tryptophan stimulates skeletal muscle IGF1/p70s6k/mTor signaling in vivo and the expression of myogenic genes in vitro. Nutrition 31: 1018-1024, 2015.
37. Durk RP, Castillo E, Marquez-Megana L, Grosicki GJ, Bolter ND, Lee CM, Bagley JR. Gut microbiota composition is related to cardiorespiratory fitness in healthy young adults. Int J Sport Nutr Exerc Metab ahead of print Jun 10, 2018. Doi: 10.1123/ijsnem.2018-0024
38. Estaki M, Pither J, Baumeister P, Little JP, Gill SK, Ghosh S, Ahmadi-Vand Z, Marsden KR, Gibson DL. Cardiorespiratory fitness as a predictor of intestinal microbial diversity and distinct metagenomic functions. Microbiome 4: 42, 2016.
39. Fadlallah J, El Kafsi H, Sterlin D, Juste C, Parizot C, Dorgham K, Autaa G, Gouas D, Almeida M, Lepage P, Pons N, Le Chatelier E, Levenez F, Kennedy S, Galleron N, Pais de Barros JP, Malphettes M, Galicier L, Boutboul D, Mathian A, Miyara M, Oksenhendler E, Amoura Z, Doré J, Fieschi C, Ehrlich SD, Larsen M, Gorochov G. Microbial ecology perturbation in human IgA deficiency. Sci Transl Med 10: eaan1217, 2018.

40. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. Science 352: 539-544, 2016.
41. Ghosh S, Lertwattanarak R, de Jesus Garduno J, Joya Galeana J, Li J, Zamarripa F, Lancaster JL, Mohan S, Hussey S, Musi N. Elevated TLR4 expression and metabolic endotoxinemia in human aging. J Gerontol A Biol Sci Med Sci 70: 232-246, 2015.
42. Greenhalgh K, Meyer KM, Aagaard KM, Wilmes P. The human gut microbiome in health: establishment and resilience of microbiota over a lifetime. Environ Microbiol 18: 2103-2116, 2016.
43. Grigg JB, Sonnenberg GF. Host-microbiota interactions shape local and systemic inflammatory diseases. J Immunol 198: 564-571, 2017.
44. Grosicki GJ. Fielding RA, Lustgarten MS. Gut microbiota contribute to age-related changes in skeletal muscle size, composition, and function: biological basis for a gut-muscle axis. Calcif Tissue Int 102: 433-442, 2018.
45. Guarner F, Malagelada JR. Gut flora in health and disease. Lancet 361: 512-519, 2003.
46. Hofmann-Goetz H, Quadrilatero J. Treadmill exercise in mice increases intestinal lymphocyte loss via apoptosis. Acta Physiol Scand 179: 289-297, 2003.
47. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science 336: 1268-1273, 2012.
48. Hsu YJ, Huang WC, Lin JS, Chen YM, Ho ST, Huang CC, Tung YT. Kefir supplementation modifies gut microbiota composition, reduces physical fatigue, and improves exercise performance in mice. Nutrients 10: 862, 2018.
49. Jackson MA, Jeffery IB, Beaumont M, Bell JT, Clark AG, Ley RE, O'Toole PW, Spector TD, Steves CJ. Signatures of early frailty in the gut microbiota. Genome Med 8: 8, 2016.
50. Kabat AM, Srinivasan N, Maloy KJ. Modulation of immune development and function by intestinal microbiota. Trands Immunol 35: 507-517, 2014.
51. Karl JP, Margolis LM, Madslien EH, Murphy NE, Castellani JW, Gundersen Y, Hoke AV, Levangie MW, Kumar R, Chakraborty N, Gautam A, Hammamieh R, Martini S, Montain SJ, Pasiakos SM. Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress. Am J Physiol Gastrointest Liver Physiol 312: G559-G571, 2017.
52. Karu N, Deng L, Slae M, Guo AC, Sajed T, Huynh H, Wine E, Wishart DS. A review on human fecal metabolomics: methods, applications, and the human fecal metabolome database. Anal Chim Acta 1030: 1-24, 2018.
53. Kobayashi Y, Hara N, Sugimoto R, Mifuji-Moroka R, Tanaka H, Eguchi A, Iwasa M, Hasegawa H, Iwata K, Takei Y, Taguchi O. The associations between circulating bile acids and the muscle volume in patients with non-alcoholic fatty liver disease (NAFLD). Intern Med 56: 755-762, 2017.
54. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, Angenent LT, Rey RE. Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci USA 108S1: 4578-4585, 2011.
55. Kriss M, Hazleton KZ, Nusbacher NM, Martin CG, Lozupone CA. Low diversity gut microbiota dysbiosis: drivers, functional implications and recovery. Curr Opin Microbiol 44: 34-40, 2018.
56. Kumar A, Davuluri G, Nascimento e Silva R, Engelen MPKJ, Ten Have GAM, Prayson R, Deutz NEP, Darasathy S. Ammonia lowering reverses sarcopenia of cirrhosis by restoring skeletal muscle proteostasis. Hepatology 65: 2045-2058, 2017.
57. Lamoreux EV, Grandy SA, Langille MGI. Moderate exercise has limited but distinguishable effects on the mouse microbiome. mSystems 2: e00006-17, 2017.
58. Lauretani F, Maggio M, Ticinesi A, Tana C, Prati B, Gionti L, Nouvenne A, Meschi T. Muscle weakness, cognitive impairment and their interaction on altered balance in elderly outpatients: results from the TRIP observational study. Clin Interv Aging 13: 1437-1443, 2018.
59. LeBlanc JG, Milani C, de Giori GS, Sesma F, van Sinderen D, Ventura M. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. Curr Opin Biotechnol 24: 160-168, 2013.
60. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. Nat Rev Immunol 17: 219-232, 2017.
61. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell 124: 837-848, 2006.
62. Lin R, Liu W, Piao M, Zhu H. A review of the relationship between the gut microbiota and amino acid metabolism. Amino Acids 49: 2083-2090, 2017.
63. Liu Z, Liu HY, Zhou H, Zhan Q, Lai W, Zeng Q, Ren H, Xu D. Moderate-intensity exercise affects gut microbiome composition and influences cardiac function in myocardial infarction mice. Front Microbiol 8: 1687, 2017.
64. Logan AC, Jacka FN, Prescott SL. Immune-microbiota interactions: dysbiosis as a global health issue. Curr Allergy Asthma Rep 16:13, 2016.
65. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature 489: 220-230, 2012.
66. Man AL, Gicheva N, Nicoletti C. The impact of ageing on the intestinal epithelial barrier and immune system. Cell Immunol 289: 112-118, 2014.
67. Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, Quraishi MN, Kinross J, Smidt H, Tuohy KM, Thomas LV, Zoetendal EG, Hart A. The gut microbiota and host health: a new clinical frontier. Gut 65: 330-339, 2016.
68. Marietta E, Rishi A, Taneja V. Immunogenetic control of the intestinal microbiota. Immunology 145: 313-322, 2015.
69. Martone AM, Marzetti E, Calvani R, Picca A, Tosato M, Santoro L, Di Giorgio A, Nesci A, Sisto A, Santoliquido A, Landi F. Exercise and protein intake: a synergistic approach against sarcopenia. Biomed Res Int 2017: 2672435, 2017.
70. Marzetti E, Calvani R, Tosato M, Cesari M, Di Bari M, Cherubini A, Collamati A, D'Angelo E, Pahor M, Bernabei R, Landi F, SPRINTT Consortium. Sarcopenia: an overview. Aging Clin Exp Res 29: 11-17, 2017.
71. McCoy KD, Ignacio A, Geuking MB. Microbiota and type 2 immune responses. Curr Opin Immunol 54: 20-27, 2018.
72. McFarlin BK, Flynn MG, Campbell WW, Stewart LK, Timmerman KL. TLR4 is lower in resistance-trained older women and related to inflammatory cytokines. Med Sci Sports Exerc 36: 1876-1883, 2004.
73. McKenzie C, Tan J, Macia L, Mackay CR. The nutrition-gut microbiome-physiology axis and allergic diseases. Immunol Rev 278: 277-295, 2017.

74. McLean MH, Dieguez jr D, Miller LM, Young HA. Does the microbiota play a role in the pathogenesis of autoimmune diseases? Gut 64: 332-341, 2015.
75. Mika A, Van Treuren W, Gonzalez A, Herrera JJ, Knight R, Fleshner M. Exercise is more effective at altering gut microbial composition and producing stable changes in lean mass in juvenile versus adult male F344 rats. PLoS One 10: e0125889, 2015.
76. Milani C, Ferrario C, Turroni F, Duranti S, Mangifesta M, van Sinderen D, Ventura M. The human gut microbiota and its interactive connections to diet. J Hum Nutr Diet 29: 539-546, 2016.
77. Milani C, Hevia A, Foroni E, Duranti S, Turroni F, Lugli GA, Sanchez B, Martin R, Gueimonde M, van Sinderen D, Margolles A, Ventura M. Assessing the fecal microbiota: an optimized ion torrent 16S rRNA gene-based analysis protocol. PLoS One 8: e68739, 2013.
78. Milani C, Mancabelli L, Lugli GA, Duranti S, Turroni F, Ferrario C, Mangifesta M, Viappiani A, Ferretti P, Gorfer V, Tett A, Segata N, van Sinderen D, Ventura M. Exploring vertical transmission of Bifidobacteria from mother to child. Appl Environ Microbiol 81: 7078-7087, 2015.
79. Molina-Molina E, Lunardi Baccetto R, Wang DQH, de Bari O, Krawczyk M, Portincasa P. Exercising the hepatobiliary-gut axis. The impact of physical activity performance. Eur J Clin Invest 48: e12958, 2018.
80. Monda V, Villano I, Messina A, Valenzano A, Esposito T, Moscatelli F, Viggiano A, Cibelli G, Chieffi S, Monda M, Messina G. Exercise modifies the gut microbiota with positive health effects. Oxid Med Cell Longev 2017: 3831972, 2017.
81. Mosca A, Leclerc M, Hugot JP. Gut microbiota diversity and human diseases: should we reintroduce key predators in our ecosystem? Front Microbiol 7: 455, 2016.
82. Neish AS. Microbes in gastrointestinal health and disease. Gastroenterology 136: 65-80, 2009.
83. Ni Lochlainn M, Bowyer MCE, Steves CJ. Dietary protein and muscle in aging people: the potential role of gut microbiome. Nutrients 10: E929, 2018.
84. Ohlsson C, Sjogren K. Effects of the gut microbiota on bone mass. Trends Microbiol 26: 69-74, 2015.
85. O'Sullivan O, Cronin O, Clarke SF, Murphy EF, Molloy MG, Shanahan F, Cotter PD. Exercise and the microbiota. Gut Microbes 6: 131-136, 2015.
86. O'Toole PW, Jeffery IB. Gut microbiota and aging. Science 350: 1214-1215, 2015.
87. Pane M, Amoruso A, Deidda F, Graziano T, Allesina S, Mogna L. Gut microbiota, probiotics, and sport. From clinical evidence to agonistic performance. J Clin Gastroenterol 52: S46-S49, 2018.
88. Pereira-Cano G, Polyviou T, Ludwig IA, Nastase AM, Moreno-Rojas JM, Garcia AL, Malkova D, Crozier A. Bioavailability of orange juice (poly)phenols: the impact of short-term cessation of training by male endurance athletes. Am J Clin Nutr 106: 791-800, 2017.
89. Petersen LM, Bautista EJ, Nguyen H, Hanson BM, Chen L, Lek SH, Sodergren E, Weinstock GM. Community characteristics of the gut microbiomes of competitive cyclists. Microbiome 5: 98, 2017.
90. Picca A, Fanelli F, Calvani R, Mulè G, Pesce V, Sisto A, Pantanelli C, Bernabei R, Landi F, Marzetti E. Gut dysbiosis and muscle aging: searching for novel targets against sarcopenia. Mediators Inflamm 2018: 7026198, 2018.
91. Ponziani FR, Zocco MA, D'Aversa F, Pompili M, Gasbarrini A. Eubiotic properties of rifaximin: disruption of the traditional concepts in gut microbiota modulation. World J Gastroenterol 23: 4491-4499, 2017.
92. Qi Y, Goel R, Kim S, Richards EM, Carter CS, Pepine CJ, Raizada MK, Buford TW. Intestinal permeability biomarker zonulin is elevated in healthy aging. J Am Med Dir Assoc 18: 810.e1-810.e4, 2017.
93. Quince C, Walker AW, Simpson JT, Loman NJ, Segata N. Shotgun metagenomics, from sampling to analysis. Nat Biotechnol 35: 833-844, 2017.
94. Rajilic-Stojanovic M, de Vos WM. The first 1000 cultural species of the human intestinal microbiota. FEMS Microbiol Rev 36: 996-1047, 2014.
95. Ramezani A, Raj DS. The gut microbiome, kidney disease, and targeted interventions. J Am Soc Nephrol 25: 657-670, 2014.
96. Relman DA. The human microbiome: ecosystem resilience and health. Nutr Rev 70: S2-S9, 2012.
97. Roberts JD, Suckling CA, Peedle GY, Murphy JA, Dawkins TG, Roberts MG. An exploratory investigation of endotoxin levels in novice long distance triathletes, and the effects of a multi-strain probiotic/prebiotic, antioxidant intervention. Nutrients 8: 733, 2016.
98. Ryu D, Mouchiroud L, Andreux PA, Katsyuba E, Moullan N, Nicolet-Dit-Felix AA, Williams EG, Jha P, Lo Sasso G, Huzard D, Aebischer P, Sandi C, Rinsch C, Auwerx J. Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents. Nat Med 22: 879-888, 2016.
99. Schmidt TSB, Raes J, Bork P. The human gut microbiome: from association to modulation. Cell 172: 1198-1215, 2018.
100. Schubert K, Olde Damink SWM, von Bergen M, Schaap FG. Interactions between bile salts, gut microbiota, and hepatic innate immunity. Immunol Rev 279: 23-35, 2017.
101. Shen S, Kumar KP, Stanley D, Moore RJ, Van TTH, Wen SW, Hickey MJ, Wong CHY. Invariante natural killer T cells shape the gut microbiota and regulate neutrophil recruitment and function during intestinal inflammation. Front Immunol 9: 999, 2018.
102. Siddhart J, Chakrabarti A, Pannérec A, Karaz S, Morin-Rivron D, Masoodi M, Feige JN, Parkinson SJ. Aging and sarcopenia associate with specific interactions between gut microbes, serum biomarkers and host physiology in rats. Aging 9: 1698-1720, 2017.
103. Sket R, Debevec T, Kublik S, Schloter M, Schoeller A, Murovec B, Vogel Mikus K, Makuc D, Pecnik K, Plavec J, Mekjavic IB, Eiken O, Prevorsek Z, Stres B. Intestinal metagenomes and metabolomes in healthy young males: inactivity and hypoxia generated negative physiological symptoms precede microbial dysbiosis. Front Physiol 9: 198, 2018.
104. Sket R, Treichel N, Debevec T, Eiken O, Mekjavic I, Schloter M, Vital M, Chandler J, Tiedje JM, Murovec B, Prevorsek Z, Stres B. Hypoxia and inactivity related physiological changes (constipation, inflammation) are not reflected at the level of gut metabolites and butyrate producing microbial community: the PlanHab Study. Front Physiol 8: 250, 2017.
105. Sket R, Treichel N, Kublik S, Debevec T, Eiken O, Mekjavic I, Schloter M, Vital M, Chandler J, Tiedje JM, Murovec B, Prevorsek Z, Linar M, Stres B. Hypoxia and inactivity related physiological changes precede or take place in absence of sig-

nificant rearrangements in bacterial community structure: the PlanHab randomized trial pilot study. PLoS One 12: e0188556, 2017.

106. Stewart LK, Flynn MG, Campbell WW, Craig BA, Robinson JP, McFarlin BK, Timmermann KL, Coen PM, Felker J, Talbert E. Influence of exercise training and age on CD14+ cell-surface expression of toll-like receptor 2 and 4. Brain Behav Immun 19: 389-397, 2005.
107. Sung MM, Byrne NJ, Robertson IM, Kim TT, Samokhvalov V, Levasseur J, Soltys CL, Fung D, Tyreman N, Denou E, Jones KE, Seubert JM, Schertzer JD, Dyck JRB. Resveratrol improves exercise performance and skeletal muscle oxidative capacity in heart failure. Am J Physiol Heart Circ Physiol 312: H842-H853, 2017.
108. Sun MF, Shen YQ. Dysbiosis of gut microbiota and microbial metabolites in Parkinson's Disease. Ageing Res Rev 45: 53-61, 2018.
109. Tana C, Lauretani F, Ticinesi A, Prati B, Nouvenne A, Meschi T. Molecular and clinical issues about the risk of venous thromboembolism in older patients: a focus on Parkinson's disease and parkinsonism. Int J Mol Sci 19(5): E1299, 2018.
110. Thaiss C, Levy M, Suez J, Elinav E. The interplay between the innate immune system and the microbiota. Curr Opin Immunol 26: 41-48, 2014.
111. Thevaranjan N, Puchta A, Schulz C, Naidoo A, Szamosi JC, Verschoor CP, Loukov D, Schenck LP, Jury J, Foley KP, Schertzer JD, Larché MJ, Davidson DJ, Verdù EF, Surette MG, Bowdish DME. Age-associated microbial dysbiosis promotes intestinal permeability, systemic inflammation, and macrophage dysfunction. Cell Host Microbe 21: 455-466, 2017.
112. Ticinesi A, Lauretani F, Milani C, Nouvenne A, Tana C, Del Rio D, Maggio M, Ventura M, Meschi T. Aging gut microbiota at the cross-road between nutrition, physical frailty and sarcopenia: is there a gut-muscle axis? Nutrients 9: E1303, 2017.
113. Ticinesi A, Milani C, Guerra A, Allegri F, Lauretani F, Nouvenne A, Mancabelli L, Lugli GA, Turroni F, Duranti S, Mangifesta M, Viappiani A, Ferrario C, Dodi R, Dall'Asta M, Del Rio D, Ventura M, Meschi T. Understanding the gut-kidney axis in nephrolithiasis: an analysis of the gut microbiota composition and functionality of stone formers. Gut 67(12): 2097-2106, 2018.
114. Ticinesi A, Milani C, Lauretani F, Nouvenne A, Mancabelli L, Lugli GA, Turroni F, Duranti S, Mangifesta M, Viappiani A, Ferrario C, Maggio M, Ventura M, Meschi T. Gut microbiota is associated with polypharmacy in elderly hospitalized patients. Sci Rep 7: 11102, 2017.
115. Ticinesi A, Tana C, Nouvenne A, Prati B, Lauretani F, Meschi T. Gut microbiota, cognitive frailty and dementia in older individuals: a systematic review. Clin Interv Aging 13: 1497-1511, 2018.
116. Ticinesi A, Tana C, Nouvenne A. The intestinal microbiome and its relevance for functionality in older persons. Curr Opin Clin Nutr Metab Care 22: 4-12, 2019.
117. Tomkovich S, Jobin C. Microbiota and host immune responses: a love-hate relationship. Immunology 147: 1-10, 2015.
118. Toohey JC, Townsend JR, Johnson SB, Toy AM, Vantrease WC, Bender D, Crimi CC, Stowers KL, Ruiz MD, Van Dusseldorp TA, Feito Y, Mangine GT. Effect of probiotic (Bacillus subtilis) supplementation during offseason resistance training in female division I athletes. J Strength Cond Res ahead of print, 2018. Doi: 10.1519/JSC0000000000002675.
119. Townsend JR, Bender D, Vantrease WC, Sapp PA, Toy AM, Woods CA, Johnson KD. Effects of probiotic (Bacillus subtilis DE111) supplementation on immune function, hormonal status, and physical performance in division 1 baseball players. Sports 6: 70, 2018.
120. Varian BJ, Goureshetti S, Poutahidis T, Lakritz JR, Levkovich T, Kwok C, Teliousis K, Ibrahim YM, Mirabal S, Erdman SE. Beneficial bacteria inhibit cachexia. Oncotarget 7: 11803-11816, 2016.
121. Vazquez-Castellano JF, Serrano-Villar S, Jimenez-Hernandez N, Soto del Rio MD, Gayo S, Rojo D, Ferrer M, Barbas C, Moreno S, Estrada V, Rattei T, Latorre A, Moya A, Gosalbes MJ. Interplay between gut microbiota metabolism and inflammation in HIV infection. ISME J 12: 1964-1976, 2018.
122. Vazquez-Castellanos JF, Serrano-Villar S, Latorre A, Artacho A, Ferrus ML, Madrid N, Vallejo A, Sainz T, Martinez-Botas J, Ferrando-Martinez S, Vera M, Dronda F, Leal M, Del Romero J, Moreno S, Estrada V, Gosalbes MJ, Moya A. Altered metabolism of gut microbiota contributes to chronic immune activation in HIV-infected individuals. Mucosal Immunol 8(4): 760-772, 2015.
123. Ventura M, Turroni F, Canchaya C, Vaughan EE, O'Toole PW, van Sinderen D. Microbial diversity in the human intestine and novel insights from metagenomics. Front Biosci 14: 3214-3221, 2009.
124. Vesterbacka J, Rivera J, Noyan K, Parera M, Neogi U, Calle M, Paredes R, Sonnerborg A, Noguera-Julian M, Nowak P. Richer gut microbiota with distinct metabolic profile in HIV infected elite controllers. Sci Rep 7: 6269, 2017.
125. Walsch ME, Bhattacharya A, Sataranatarayan K, Qaisar R, Sloane L, Rahman MM, Kinter M, Van Remmen H. The histone deacetylase inhibitor butyrate improves metabolism and reduces muscle atrophy during aging. Aging Cell 14: 957-970, 2015.
126. Wilson D, Jackson T, Sapey E, Lord JM. Frailty and sarcopenia: the potential role of an aged immune system. Ageing Res Rev 36: 1-10, 2017.
127. Yang Y, Shi Y, Wiklund P, Tan X, Wu N, Zhang X, Tikkanen O, Zhang C, Munukka E, Cheng S. The association between cardiorespiratory fitness and gut microbiota composition in premenopausal women. Nutrients 9: 792, 2017.
128. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP, Heath AC, Warner B, Reeder J, Kuczynski J, Caporaso JG, Lozupone CA, Lauber C, Clemente JC, Knights D, Knight R, Gordon JI. Human gut microbiome viewed across age and geography. Nature 486: 222-227, 2012.
129. Yuan X, Xu S, Huang H, Liang J, Wu Y, Li C, Yuan H, Zhao X, Lai X, Hou S. Influence of excessive exercise on immunity, metabolism, and gut microbial diversity in an overtraining mice model. Scand J Med Sci Sports 28: 1541-1551, 2018.
130. Zhang H, Luo XM. Control of commensal microbiota by the adaptive immune system. Gut Microbes 6: 156-160, 2015.
131. Zhao X, Zhang Z, Hu B, Huang W, Yuan C, Zou L. Response of gut microbiota to metabolite changes induced by endurance exercise. Front Microbiol 9: 765, 2018.
132. Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, Vatanen T, Mujagic Z, Vila AV, Falony G, Vieira-Silva S, Wang J, Imhann F, Brandsma E, Jankipersadsing JA, Joossens M, Cenit MC, Deelen P, Swertz MA, LifeLines Cohort Study, Weersma RK, Feskens EJ, Netea MG, Gevers

D, Jonkers D, Franke L, Aulchenko YS, Huttenhower C, Raes J, Hofker MH, Xavier MJ, Wijmenga C, Fu J. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science 352: 565-569, 2016.

133. Zilberman-Schapira G, Zmora N, Itav S, Bashiardes S, Elinav H, Elinav E. The gut microbiome in human immunodeficiency virus infection. BMC Med 14: 83, 2016.
134. Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S, Kotler E, Zur M, Regev-Lehavi D, Brik RBZ, Federici S, Cohen Y, Linevsky R, Rothschild D, Moor AE, Ben-Moshe S, Harmelin A, Itzkovitz S, Maharshak N, Shibolet O, Shapiro H, Pevsner-Fischer M, Sharon I, Halpern Z, Segal E, Elinav E. Personalized gut mucosal colonization resistance to empiric probiotics is associated with unique host and microbiome features. Cell 174: 1388-1405, 2018.

Neuroimmunological and Neuroenergetic Aspects in Exercise-Induced Fatigue

Sebastian Proschinger[1] and Jens Freese[2]

[1] German Sport University Cologne, Department for Molecular and Cellular Sports Medicine, Am Sportpark Müngersdorf 6, 50933 Cologne, Germany
[2] Dr. Freese Institute for Exercise & Nutritional Immunology, Josef-Lammerting-Allee 7-13, 50933 Cologne, Germany

Abbreviations

ADO	–	adenosine
ATP	–	adenosine triphosphate
BBB	–	blood-brain barrier
CNS	–	central nervous system
CVO	–	circumventricular organs
DAMP	–	danger-associated molecular pattern
GABA	–	γ-aminobutyric acid
IL-1	–	Interleukin 1
IL-6	–	Interleukin 6
LPS	–	lipopolysaccharide
PAMP	–	pathogen-associated molecular pattern
RNS	–	reactive nitrogen species
ROS	–	reactive oxygen species
S100	–	S100 calcium-binding protein
TNF	–	tumor necrosis factor-alpha
TLR	–	Toll-like receptor
VO_{2max}	–	maximal oxygen consumption
5-HT	–	5-hydroxytryptamine

Index

* *Corresponding author:*
Sebastian Proschinger[1], s.proschinger@gmx.net
Jens Freese[2], info@dr-freese.com

ABSTRACT

Feelings of fatigue not only occur in chronic and acute disease states, but also during prolonged strenuous exercise as a symptom of exhaustion. The underlying mechanisms of fatigue in diseases seem to rely on neuroinflammatory pathways. These pathways are interesting to understand exercise-induced fatigue regarding immune system to brain signaling and effects of cerebral cytokines. Activation of the immune system incurs a high-energy cost, also in the brain. In consequence immune cells have high energetic priority over other tissues, such as neurons. A neuronal inactivation and corresponding changes in neurotransmission can also be induced by end products of ATP metabolism and elicit feelings of fatigue in diseases and after intensive and prolonged exercise bouts. Since there are no existing models of exercise-induced fatigue that specifically address interactions between neuroimmunologic mechanisms and neuroenergetics, this article is combining scientific evidence across a broad range of disciplines in order to propose an inflammation- and energy-based model for exercise-induced fatigue.

Keywords: exercise-induced fatigue, neuroinflammation, neuroenergetics, adenosine, cytokines.

1. Introduction

To study exercise-induced fatigue for many years, priority was given to muscles over the brain as a regulatory factor. Already in 1915, Alessandro Mosso postulated that both, the will (central component) and the muscular work (peripheral component), have to be taken into account when considering the resulting impairment of exercise performance. Mosso distinguished the diminution of the muscular force and the sensation of fatigue (1). As a result of the upcoming knowledge of the bi- and multidirectionality of biological systems, the paradigm shifted to the inclusion of cerebral processes in order to guarantee homeostasis in all systems during exercise by modulating athlete´s behavior (2,3).

Since proinflammatory cytokines induce changes in behavior during acute infection by provoking feelings of fatigue (4,5), it is reasonable that the remarkable rise in circulating proinflammatory signals during prolonged strenuous exercise (6) may also contribute to exercise-induced fatigue. In this regard, the neuromodulatory properties of myogenic/neuronal Interleukin 6 (IL-6) and cerebral immune cell-derived Interleukin 1 (IL-1) have recently been discussed as major factors in exercise-induced fatigue (7,8).

According to the selfish immune system theory (9), high synthesis rates of cytokines indicate high energy turnover of immune cells and with that, higher energetic needs of those. In the case of an increasing brain macrophage activity, energy substrates may be shifted away from neurons to these immune cells to maintain their activity (8,10). Because decreasing neuronal activity seems to induce feelings of fatigue also during exercise, a compromised energy provision to neurons due to increasing brain immune cell activity could account for the decline in exercise performance (11,12).

The initially increasing neuronal and glial energy turnover during prolonged strenuous exercise (11,12) may favor the generation of the nucleoside adenosine (ADO) (13), which negatively mediates exercise performance in a concentration-dependent manner by modulating dopamine neurotransmission in the basal ganglia (14,15).

Here, we propose that (neuro-)immunological mechanisms influence neuroenergetics, with both proinflammatory signals and end products of energy turnover inducing feelings of fatigue during prolonged strenuous exercise and ultimately provoking exercise termination.

2. Peripheral and central fatigue

Already in the late nineteenth century, the physiologist Angelo Mosso postulated that "muscular fatigue also is at bottom an exhaustion of the nervous system" (2). In the context of exercise-induced fatigue, central or supraspinal fatigue appears to originate in regions of the brain and is defined as the inability of the CNS to drive motor neurons efficiently during the performance of intermittent or prolonged aerobic exercise (16), whereas peripheral or muscle fatigue is the result of biochemical changes in the exercising limb muscles (17).

2.1 Lactate accumulation

According to the lactate theory of exercise fatigue, the exercising muscles stop working due to a massive intracellular lactate accumulation as a consequence of an insufficient supply of oxygen and the upregulation of the muscle cell´s anaerobic metabolism (17).

However recent findings challenge the correctness of the lactate theory (Robergs, 2004) and emphasize the significance of lactate as energy substrate in other metabolic processes (18,19). Via intracellular monocarboxylate transport proteins, lactate is used as an additional energy substrate both by contracting and adjacent inactive muscle fibers. During strenuous exercise, a reciprocal brain-muscle energy exchange occurs in which the brain favors muscle-derived lactate in order to provide enough circulating glucose to type-2 muscle fibers as its primary energy substrate (20-22).

The energetic capacity of exercising muscles does not decrease significantly to promote peripheral fatigue, since muscles are still capable to generate power at exhaustion (23). Because neither lactate accumulation in exercising muscles nor associated muscle acidification cause peripheral fatigue (23), these findings underline the assumption of exercise termination forced by central mechanisms.

2.2 Neurotransmission

Neurotransmission of monoamines plays a crucial role in exercise-induced fatigue. The central fatigue hypothesis postulated by Newsholme et al. (24) states that exercise-induced synthesis of cerebral serotonin (5-HT) provokes the onset of fatigue symptoms. Since 5-HT can not cross the blood-brain barrier (BBB), brain cells rely on the uptake of tryptophan as its precursor. Animal studies (25) have shown that tryptophan injections in the cerebral ventricle of rats were associated with the onset of exercise-induced fatigue, while inhibition of the conversion of tryptophan to 5-HT could improve running time to fatigue. However, others have proven a reduction in plasma tryptophan in humans after exhaustive aerobic exercise (26), which seems contradictory to the aforementioned findings. Strasser et al. conclude that there is limited availability of tryptophan for 5-HT biosynthesis in the brain after the enzymatic conversion to kynurenine in the periphery.

Recent findings provide evidence that dopaminergic neurotransmission in striatopallidal neurons increases exercise performance by maintaining motivation and motor regulation (27,28). A blockage of central dopaminergic D1/D2 receptors results in a significant decrease in endurance performance and maximal oxygen uptake (29).

2.3 Cytokines

Many systemic inflammatory and neuroinflammatory disorders, i.e. chronic fatigue syndrome (CFS), depression or multiple sclerosis, are frequently accompanied by high amounts of circulating cytokines and a persistent state of mental and physical fatigue (30). Neuroimaging studies have suggested the presence of neuroinflammation in the midbrain of CFS patients (31). Furthermore, CFS patients achieve volitional exhaustion significantly faster and consistently report a higher rate of perceived exertion during an exercise task, assuming that CFS, in part, is mediated centrally (32). Chronic fatigue in athletes suffering from overtraining/athlete burnout may also result from circulating proinflammatory cytokines and a neuroinflammatory state (33,34).

Vargas & Marino (35) proposed a neuroinflammatory model for acute fatigue during exercise. The authors suppose a potential interaction between cytokine release during prolonged strenuous exercise and their effects on afferent feedback signalling to the brain that might lead to feelings of fatigue. In particular, the extraordinary increase in plasma IL-6 concentration is proposed to be a major fatigue-inducing factor due to its receptor-mediated signal transduction in neuronal afferents and circumventricular organs (CVO).

Already in 2000, the influence of muscle-derived IL-6 was considered to play an important role in the development of central fatigue (36). Subcutaneous administration of a low dose of recombinant IL-6 to athletes increase their sensation of fatigue at rest and significantly impairs athletic performance during a 10-km running time trial (37). Because of its autocrine, paracrine or endocrine effects, muscle-derived IL-6 may also function as an energy sensor and a hormone-like molecule that increases energy substrate mobilization (38-40), possibly by an intensity-dependent upregulation of cortisol (41,42). Therefore, high IL-6 levels could represent the need for energy substrates.

After an eccentric exercise bout, the concentration of IL-1 increases significantly in rat brain regions responsible for movement, coordination, motivation, perception of effort, and pain. Its levels correlate significantly with both post-exercise delayed recovery and decreased performance in a subsequent task (43). Further, intracerebroventricular injection of IL-1 significantly decreased wheel running activity in uphill running mice, whereas IL-1ra improved wheel running in downhill running mice (44). Another study identified perivascular and meningeal macrophages as the major producer of brain IL-1 during exercise (8).

There is vast evidence that microglia, another mononuclear phagocytic cell type in the CNS and the main actor in neuroinflammation, synthesize both IL-1 and TNF in high amounts after activation. Furthermore, the decrease in symptoms of depression and fatigue is accompanied by a reduced TNF secretion in the CNS through modulation of neuroinflammation (31,45,46).

3. Systemic inflammatory response during exercise – muscle damage, leukocytosis and endotoxemia

Via the production of IL-6 and reactive oxygen species (ROS), both exercise-induced muscle damage (47,48) and the intensity-dependent rise in circulating T-lymphocytes and neutrophils (49,50) significantly contribute to the exercise-induced systemic inflammation (51,52). The rise in serum neopterin during exhaustive aerobic exercise suggests an increased activation of peripheral macrophages (26). However, results from Ostrowski et al. (53) reveal an increase of the anti-inflammatory cytokines IL-10, IL-1 receptor antagonist and soluble TNF receptors during and after strenuous exercise, possibly due to the massive increase in IL-6 (41,54).

Lymphocyte-derived extracellular heat shock proteins are known to increase during high-load exercise and are further proposed to promote fatigue sensation via marked influence on motor neurons and deeper structures of the CNS (55). These molecules also promote inflammation by acting as a danger signal from the immune system. Bårdsen et al. (56) suggest that the significant increase in extracellular heat shock proteins in CFS patients might signal to the brain and contribute to the state of fatigue.

The observation that prolonged strenuous exercise favors a systemic inflammatory state was discussed by John Marshall, assuming that the exercise-induced increase in intestinal permeability and lipopolysaccharide (LPS)-induced endotoxemia may be the underlying cause (57). LPS is a gut-derived proinflammatory fragment of the outer membrane of gram-negative bacteria and a pathogen-associated molecular pattern (PAMP). Pals et al. (58) showed that the degree of the intestinal permeability depends mainly on exercise intensity and correlates with body core temperature. In fact, human studies show that the severity of endotoxemia seems highly dependent on the environmental temperature (59,60), but also on the composition of the gut microbiota (61). In this regard, the supplementation of probiotics over a period of 4 weeks displays a tendency to decreasing intestinal permeability and reducing LPS in the bloodstream (62).

After an ultramarathon, 81% of the participants showed plasma LPS levels > 0.1 ng/ml (endotoxic), while 2% even had a plasma concentration of 1 ng/ml (potentially lethal) despite moderate environmental temperatures (20,3°C-22,3°C) (63). Both exercise-induced functional splanchnic hypoperfusion and translocation of LPS are damaging the protein-barrier complex between enterocyte membranes via temperature-dependent and immune-mediated mechanisms (64-66). This contributes to an endotoxic state.

4. Communication interfaces between periphery and central nervous system

A systemic inflammatory response has been shown to affect the activity of immune cells in the brain. The growing importance of the bidirectionality between the periphery and the central nervous system (CNS) and the impact of neuroimmunomodulatory mechanisms (67) puts the interplay of endocrine, neuronal and immunological mechanisms in the forefront of exercise regulation (3). Due to acute or chronic immune stressors, dysregulation at periphery-CNS interfaces, i.e. the BBB, CVO, and afferent nerve fibres (68), is associated with pathological conditions in which fatigue is a common feature (69). As prolonged strenuous exercise represents a huge physiological stressor accompanied by immune activation, interface-specific cells could get regulated in order to induce systemic adaption and maintain homeostasis in all systems during exercise (2,70).

Some cytokines use specific mechanisms to access the brain parenchyma by bypassing its saturable transport mechanisms (71). The serum level of the S100 calcium-binding protein (S100) which provides information about the severity of the BBB´s permeability, increases during strenuous exercise (72). Both duration and intensity of an exercise bout (73) and game-related activities or events (74) seem to determine the rise in S100 plasma concentration. Furthermore, S100 is the most frequently assessed biomarker in studies investigating sport-related concussion which is known to induce BBB disruption (75). According to the severity of concussion, the post-injury neuroinflammatory state promotes metabolic dysfunction and neuronal impairment (76), often followed by persistent feelings of fatigue, without regard of traumatic brain injury severity (77,78). A correlation between the onset of exercise-induced fatigue and the number or magnitude of impacts to the head is possible, but experimental data are lacking.

Although LPS is able to alter transport rates for many peptides across the BBB (79), LPS acts on receptors outside the BBB rather than directly on BBB´s structures to modulate its integrity (80,81). Peripheral administration of subseptic doses of LPS initiates the synthesis of IL-1 and tumor necrosis factor-alpha (TNF) messenger RNA at the CVO, but not at the BBB (82). Since plasma LPS concentration can rise significantly during prolonged strenuous exercise (63), CVO could play a decisive role in neuroimmunological modulation. Recent studies show that communication between peripheral immune cells and brain structures predominantly occurs at the sensory CVO (83). Their unique structure enables them to

monitor and transmit blood- and cerebrospinal fluid-derived information from circulating substances that do not readily cross the BBB.

During a systemic inflammatory response, concentrations of the IL-6 receptor and the IL-6 signal transducer glycoprotein 130 are highest in the sensory CVO. The synthesis rate of both increase significantly in accordance with circulating IL-6 (84), thereby enforcing its neuroimmunomodulatory properties. The huge rise in serum IL-6 during prolonged strenuous exercise may increase levels of soluble IL-6 receptor and glycoprotein 130 in the sensory CVO. A systemic inflammatory response upregulates IL-1 receptor and TLR (Toll-like receptor) 4 in the sensory CVO as well, both changing the activity of neurons and inducing gene expression of proinflammatory cytokines (85-87). The IL-1 receptor and TLR4 is expressed by microglia and by brain macrophages. After a single systemic administration of LPS, microglia show increased proliferation in the sensory CVO compared with other regions of the brain (88), presumably compensating for the lack of a protecting BBB.

Receptors for cytokines and LPS are also expressed at the terminal nerve endings of the vagus nerve, suggesting a crucial role in immunomodulation and sickness behavior via signalling from nucleus tractus solitarius to brainstem, hypothalamus and higher brain centers (89-91). Once these receptors become activated, the vagus nerve is stimulated in a dose-dependent relationship (92,93).

Since the afferent activity of the hepatic vagus nerve seems to contribute to the orchestration of the metabolic and hormonal responses to exercise, cytokine-induced stimulation of the vagus nerve could influence exercise performance in a dose-dependent manner (94). Similarly, activation of glial cells in the spinal cords of mice during eccentric exercise alters their gene expression due to the emerging skeletal muscle inflammation (95), provoking exercise-induced muscle hyperalgesia by IL-6 signalling on primary afferent nociceptors (96). Enhanced glial cytokine synthesis in the spinal cord is also shown during acute and chronic pain states and in inflammatory muscle disease (97,98) with fatigue and pain pathways being quite similar regarding cytokine signalling (99).

5. Neuroinflammation and fatigue

Since the perception of fatigue as a hallmark of sickness behavior seems to be cytokine-driven (4,5), fatigue is widespread in people suffering from neurodegenerative and chronic inflammatory diseases (30). Both direct and indirect measurement methods revealed an increased intestinal permeability, higher circulating LPS levels and a region-specific rise in neuroinflammation (100-104). Therefore, a causal relationship between intestinal permeability, neuroinflammation and the perception of fatigue is reasonable.

Rats exposed to either an immunological or a physical stressor show symptoms of sickness behavior in a time-dependent manner. However, when IL-1 receptor antagonist is injected intracerebroventricularly prior to the physical stress exposure, symptoms do not appear (105). Indeed, IL-1 and IL-6 may function as immunological correlates of human sickness behavior. During infection, levels of IL-1 and IL-6 spontaneously released from peripheral blood mononuclear cell cultures were consistently correlated with reported manifestations of acute sickness behavior including fever, malaise, pain, fatigue, mood and poor concentration (106).

An animal study showed that the administration of anti-inflammatory omega-3 fatty acids significantly inhibit LPS-induced neuroinflammation in the prefrontal cortex, hippocampus and hypothalamus and reverses depression-like behavior (46). Moreover, supplementation of the omega-3 fatty acid eicosapentaenoic acid in the course of 16 weeks promotes symptom remission and structural brain changes in patients with CFS (107).

6. Energetic regulation – is there a selfish immune system in the brain?

From an ecoimmunological point of view, an acute inflammatory response is metabolically extremely costly according to its allostatic load (108). As allostasis is an evolutionarily conserved and energy-intensive response to resume local homeostasis, the allostatic load indicates the severity of the homeostatic disruption (109). Based on in vitro O2-consumption rates (24), activated macrophages turn over ATP ten times faster per minute compared to the inactivated state. The favored aerobic glycolysis of activated immune cells makes glucose their primary energy substrate (110), using strategies to redistribute energy to themselves to keep their metabolism running (9). New insights indicate that these characteristics can also be observed in microglia depending on their polarization state (10,88,111).

Assuming that brain macrophages become overactive during prolonged strenuous exercise (8), their energy needs could reduce energy provision to neurons, thereby promoting the occurrence of fatigue symptoms. In patients with tuberculous meningitis, the infection with Mycobacterium tuberculosis represents a huge allostatic load indicated (112). The infection is accompanied by microglial activation and the allocation of astrocytic lactate to microglia via astrocyte-microglia lactate shuttles, thereby providing an adequate energy supply activated microglia. As a result, the allocation of lactate to neurons decreases significantly, which leads to neuron inactivation (10). Similarly, when lactate shuttling from astrocytes to neurons decreases during strenuous exercise, neurons are not able to maintain their metabolism (11,113). In consequence, exercise performance declines.

Acute bouts of strenuous exercise mobilize highly differentiated T-cells from peripheral tissues into the blood stream (49,114) referring to exercise-induced leukocyte demargination (115). Since a high differentiation level is associated with decreased mitochondrial content and function, these immune cells mainly rely on the glucose-consuming anaerobic metabolism (116). The trafficking rate depends on the aerobic fitness level with untrained people showing higher redistribution of these energy consuming immune cells into the blood

stream (117,118), potentially contributing to the earlier onset of exercise-induced fatigue in this population.

A high energy turnover induces ATP breakdown to ADO. ADO is secreted by ATP-depleted tissues or is extracellularly generated from ATP, which is released from metabolically stressed cells (119). In a Drosophila infection model, ADO induces energy reallocation by enhancing uptake of glucose in immune cells at the expense of other glucose-dependent tissues, including the brain (120). Consequently, ADO is considered being a signalling molecule whose effects could increase fatigue in relation to the energetic demand of activated immune cells (121). It is important to note that ADO regulatory and signaling network in Drosophila is similar to mammalian systems (121). Since high levels of ADO accumulate in the brain after prolonged strenuous exercise (13), it is reasonable that there could be similar mechanisms of action.

7. Purinergic regulation of neuroinflammation and neurotransmission in the basal ganglia

New insights into mechanisms of action of purines in the CNS with respect to neuroinflammatory processes and behavioral regulation emphasize their neuromodulatory effects, although most results are from animal studies (122). A rise in extracellular ADO favors neuroinflammatory signalling through upregulation of the high-affinity A_{2A} adenosine receptor (123). As high amounts of extracellular ATP are considered to be evolutionarily conserved danger-associated molecular pattern (DAMP) (124), it initiates inflammation via stimulation of the TLR4-dependant cytosolic inflammasome in microglia (125). While both ADO and ATP are able to enhance the production of IL-1 (126), IL-1 in turn promotes ATP and ADO release from neurons (127). Experimental data in mice suggest a potentiation of nitric oxide release by activated microglia after interacting with the A_{2A} adenosine receptor, thereby increasing ROS and reactive nitrogen species (RNS) production (128-130). In addition, the stimulation of the ATP-purinoceptors P2X7R and P2X4R favors synthesis of IL-6 and TNF, what further promotes neuroinflammation (131).

ADO directly influences behavior by decreasing dopaminergic neurotransmission through conformational changes of D2R binding sites at a shared A_{2A}/D_2- and $A_{2A}/D_2/mGlu_5$-receptor complex on rat striatopallidal GABA neurons (15,132,133). As dopamine is an important neurotransmitter in exercise regulation, ADO may negatively influence exercise performance in rats (134). In contrast, the ADO antagonist caffeine delays run time to fatigue in rats by 52%, presumably by increasing dopamine release through an antagonism at the A_1 and A_{2A} adenosine receptors in the striatum, the nucleus accumbens and the nucleus caudatus (135) or the preoptic area and the anterior hypothalamus (136). However, no effect of caffeine on exercise performance was seen in humans exercising in high ambient temperature (137).

8. Neuroinflammation-induced energy reallocation during exercise – a new paradigm?

Not only exercise-induced muscle damage, endotoxemia and leukocytosis contribute to the systemic inflammatory response in exhausted athletes, but also the release of ROS/RNS and, to a lower extent, cytokine-dependent apoptosis of leukocytes and neutrophils immediately after prolonged strenuous exercise (138,139). Although circulating lymphocytic subpopulations contain a high antioxidant capacity (140), it is conceivable that leukocytes whose capacity has already been exhausted during prolonged strenuous exercise could undergo apoptosis even before exercise termination. Cells that are not immediately phagocytosed after apoptosis become “leaky” (secondary necrosis). They release DAMPs and stimulate a host response by secreting more proinflammatory signals (141).

Exercise-induced rise in serum LPS concentration may induce changes at the BBB and favors microglia proliferation at the CVO, thereby inducing neuroinflammation (80,88). If gut-derived LPS accumulates in the liver by overwhelming the capability of the liver´s reticulo-endothelial system (63), the resulting stimulation of Kupffer cells may force the secretion of cytokines. Binding of LPS and IL-1 to receptors on terminal nerve endings of the hepatic vagus nerve may activate microglia (69,142).

There is some evidence that IL-6 acts as a major factor and is contributing to exercise-induced fatigue (7,36,37). Results from prolonged (marathon) and highly prolonged (spartathlon) endurance exercise show a 128-fold and respectively 8000-fold increase in IL-6 plasma levels, peaking at exercise termination and rapidly normalizing afterwards (53,143). This outcome may support the fatigue-inducing character of IL-6 instead of being a proinflammatory cytokine in the context of exercise. However, as energy availability declines drastically due to the physical strain in such events, muscle-derived IL-6 may also work in its hormone-like fashion by increasing energy substrate mobilization (38-40).

Since increased neuronal metabolism alters microglia functioning, neurons can be regarded as key immune modulators in the brain (144). As neuronal metabolism and extracellular levels of ‘neuron-microglia signalling factors’ rise, they function as "On" signals (Fig. 1: right box, dark blue arrows) by recruiting microglia which then support the neuron´s metabolism (Fig. 1: microglia-astrocyte-neuron lactate shuttles = right box, purple arrow). Already before, astrocytes begin to serve the energy needs of the neurons through cellular lactate transfer (Fig. 1: astrocyte-neuron lactate shuttles = light blue arrow).

The rise in extracellular ADO due to the high glial and neuronal ATP turnover may increase astrocyte proliferation and activation (145). The significant increase in brain ADO during strenuous exercise (13), could, therefore, aim to enhance astrocytic lactate production to supply the cells in need (Fig. 1). Furthermore, cerebral ADO modulates BBB permeability through stimulation of endothelial $A_{2A}R$ and A_1R (146). An enhanced uptake of blood lactate may be the consequence, as

a moderately increased permeability of the BBB is regarded as a functional mechanism during exercise by serving neuronal metabolism (27). Marked changes, however, could may limit the individual's capacity to perform optimally by allowing the accumulation of unwanted substances in the CNS (27).

Because almost all metabolic processes show a dose-response relationship during stress exposure with both beneficial and detrimental outcomes (147), exercise above a certain threshold can cause mal-adaptations as well (148). Regarding prolonged strenuous exercise, the exercise-related dose response induces an inflammatory state (Fig. 1) and may also provoke an acute neuroinflammatory response (8) due to the high allostatic load on brain cell metabolism. However, experimental data are lacking to make clear conclusions about brain metabolism during exercise and its relation to neuroinflammation. But to integrate the existing knowledge about exercise-related dose response into the concept of neuroinflammation, we propose a model of continuum in which the astrocyte-neuron lactate shuttle expands to the microglia-astrocyte-neuron lactate shuttle (149) when energy demand of neurons increase during exercise (Fig. 1, right box). Both, the intensity-dependent systemic inflammatory response and brain cell-derived purines may switch the microglial phenotype from the M2/anti-inflammatory form to the M1/proinflammatory form, thereby making them more "energy-craving". That is followed by a step-by-step inactivation of neurons through astrocyte-microglia lactate shuttles (10) (Fig. 1: right box, red arrow).

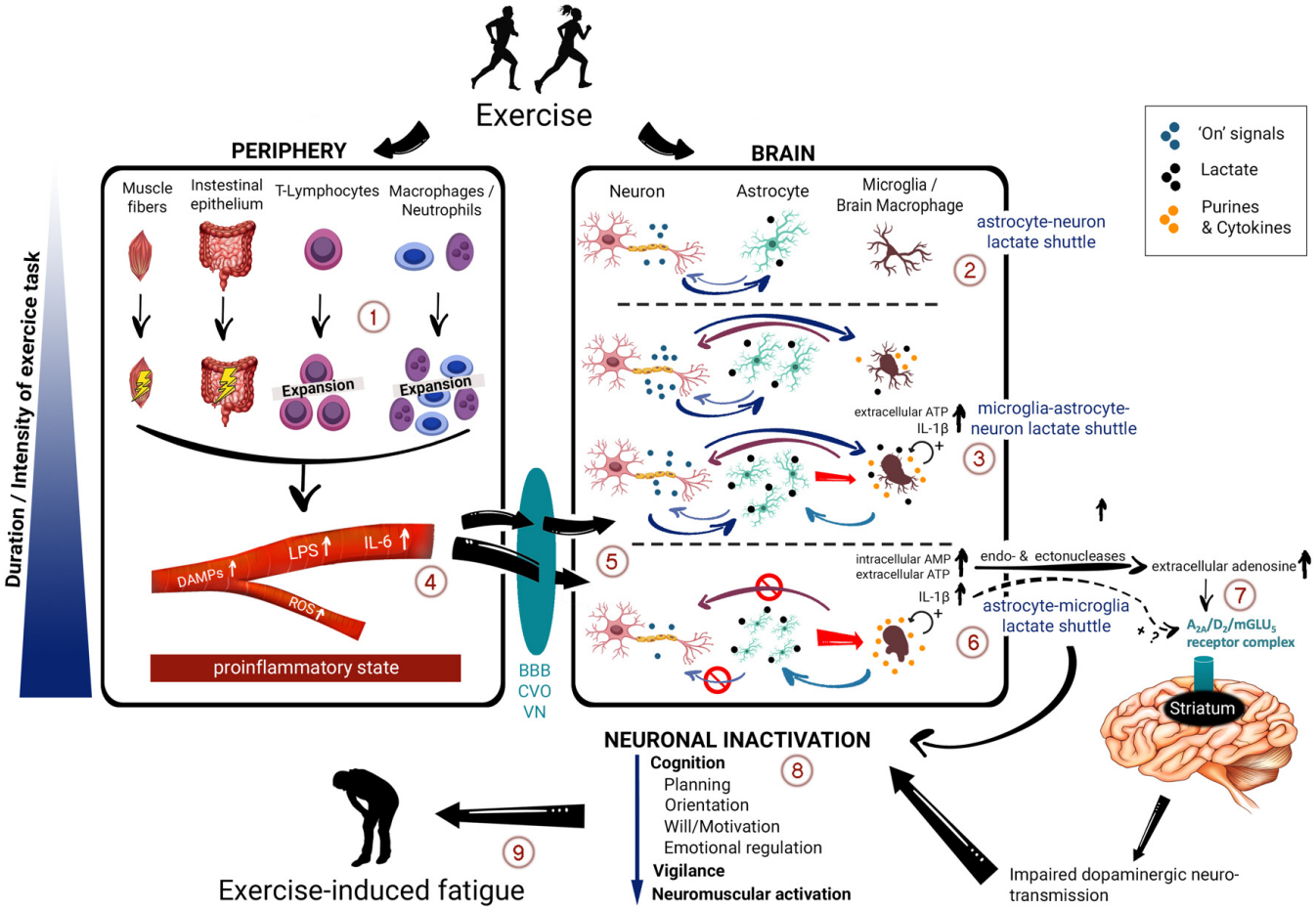


Figure 1: Hypothetical integrative model showing how neuroimmunological and neuroenergetic mechanisms induce feelings of fatigue during prolonged strenuous exercise, ultimately provoking exercise termination.
Strenuous exercise favors exercise-induced muscle damage, gut-derived LPS translocation and immune cell expansion (leukocytosis) [1]. At the same time, the exercise-induced and intensity-dependent increase in neuronal metabolism favors the release of neuronal 'On'-signals, which induce lactate transfer from glial cells to neurons, beginning with the astrocyte-neuron lactate shuttle [2] and extending to the microglia-astrocyte-neuron lactate shuttle in order to serve the increasing energy needs of the neurons [3]. Microglial autoactivation through microglia-derived IL-1β and extracellular ATP may promote a switch to the M1/proinflammatory form. As strenuous exercise continues, that leads to a proinflammatory state characterized by high circulating amounts of LPS, DAMPs (e.g. HSPs), IL-6 and ROS-damaged immune cells. These proinflammatory signals act either on the BBB, CVOs and VN which then signal to the CNS or they act directly on the CNS by passing the BBB or CVOs [4]. In doing so, these signals may stimulate microglia/brain macrophages through TLR-4 and IL-1β receptors [5] which then continuously shut down the lactate transfer from astrocytes to neurons through a yet unknown ("selfish"?) mechanism in order to benefit most from astrocytic lactate [6]. Further, the degradation of ATP and AMP through endo- and ectonucleases favor accumulation of extracellular adenosine that impairs dopaminergic neurotransmission by acting on the A2A/D2/mGLU5 receptor complex on striatal neurons [7]. A possible contribution of IL-1β to adenosine signalling may further enhance the down regulation of dopaminergic neurotransmission. The resulting neuronal inactivation [8] leads to a decline in cognition, vigilance and neuromuscular activation, ultimately inducing exercise-induced fatigue [9].
A_{2A}: adenosine A_{2A} receptor; D_2: dopamine D_2 receptor; $mGLU_5$: metabotropic glutamate receptor 5; IL-1β: interleukin 1β LPS: lipopolysaccharide; IL-6: interleukin 6; DAMP: danger-associated molecular patterns; BBB: blood-brain barrier; CVO: circumventricular organs; VN: vagus nerve; AMP: adenosine monophosphate; ATP: adenosine triphosphate; TLR-4: toll-like receptor 4; HSP: heat shock protein; CNS: central nervous system.

This microglial polarization is often accompanied by a shift from oxidative phosphorylation to aerobic glycolysis for energy production due to increasing concentrations of nitric oxide by inducible nitric oxide synthetase which reversibly inhibits mitochondrial respiration (111). With that, ROS and RNS production is increased which, in turn, activates downstream signaling pathways resulting in the up-regulation of a variety of proinflammatory proteins and more ROS/RNS.

Whether there is a similar mechanism of energy reallocation from neurons to activated microglia/brain macrophages during non-infectious stress is unknown. However, haemodynamically stressed microglia express monocarboxylate transporter-1 and -2 (150), which may enable them to utilize astrocytic glycogen-derived lactate. Since there is remarkable cerebral haemodynamic stress during prolonged strenuous exercise (151), expression of monocarboxylate transporters may promote the uptake of astrocytic lactate in microglia or brain macrophages.

Although the amount of LPS crossing the BBB is low (80,81), some athletes show plasma concentration of 1 ng/ml after an ultramarathon (63). If LPS crosses the BBB at that concentration is unknown, but conceivable since the BBB becomes leakier during strenuous exercise. As LPS-TLR4 interactions resemble proinflammatory pathways induced by Lipoarabinomannan, the major cell wall component of mycobacterium tuberculosis (152,153), high amounts of LPS in the brain may be able to induce the expression of astrocyte-microglia lactate shuttles. Further, cerebral DAMPs may promote astrocyte-microglia lactate shuttles in a similar fashion by triggering the microglial TLR4 (Fig. 1).

Heck et al. (55) propose that the exercise-induced increase in circulating levels of extracellular 70-kDa heat shock proteins from lymphocytes promote fatigue via marked influence on motor neurons and deeper structures of the CNS. Although specific receptors for heat shock proteins in brain tissue have not been identified yet, their ability to induce proinflammatory signalling in TLR4/2-expressing cells is well established (154,155).

Because lactate does not accumulate in cerebrospinal fluid after an exhaustive exercise task (156), unlike during tuberculous meningitis (157), we do not know whether it is appropriate to think of the astrocyte-microglia lactate shuttles as a relevant mechanism in exercise-induced fatigue. Further it is unknown whether extracellular ADO reallocates energy substrates to demanding cerebral immune cells and thereby shutting down the less relevant neuronal metabolism as shown in a Drosophila infection model on the peripheral level. Extracellular ADO definitively compromises exercise performance in animals due to its inhibitory effect on dopaminergic neurotransmission (134,135). To connect the potential fatigue-inducing property of ADO, Hanff et al. (158) assume that it plays an important role in the induction of sickness behavior via the A2A/D2/mGLU5-receptor-complex (Fig. 1). In fact, LPS-induced swim deficits is reversed by systemic administration of an A2A receptor antagonist (159). A similar receptor-ligand interaction appears to be relevant in the induction of sleep (160). The stimulation of A2AR and mGLU5R inhibits the activity in vigilance-regulating brain areas by presynaptic inhibition, postsynaptic hyperpolarization and amplifying GABAergic projections (161,162). Increased dopamine release in the ventral tegmental area reduces the inhibitory activity in the nucleus accumbens and is promoting vigilance.

Dopaminergic neurotransmission in the substantia nigra pars compacta inhibits neuroinflammation by activating astrocytic D_2-receptors (163). Based on the assumption that IL-1 may contribute to motivational and vigilance regulation via an important interaction with ADO signalling in the CNS, i.e. activation of A2A receptors in the striatum (158) (Fig. 1), the attenuated dopamin-induced anti-inflammatory effect could promote synthesis of IL-1. Both, inflammation- and exercise-induced peripheral hyperammonaemia promote cerebral synthesis of ADO (164,165), which may force exercise-induced fatigue by altering cognition (165). The increasing impairment of the fronto-striatal network down-regulates cognition and motivation, which makes exercise termination rather a relative than an absolute event due to the athlete´s volitional and forced conscious decision (see Fig. 1) (3,166,167). The impact of peripheral cytokine signalling and central microglia/brain macrophage activation on this fronto-striatal network should be taken into account (69).

CONCLUSION

Exercise-induced fatigue does not emerge from a single peripheral or central mechanism, but rather result of a synergistic effect of various mechanisms involving both peripheral and central aspects. As an evolutionary conserved protective mechanism, neuron inactivation and the concomitant increase in feelings of fatigue are extremely useful to maintain systemic homeostasis at all bodily levels, also during exercise. If the immune system is even selfish in the brain, microglia/brain macrophage-derived extracellular ADO could mediate the metabolic switch and energy reallocation, thereby inducing neuron inactivation, feelings of fatigue and ultimately exercise termination. Due to the impact of IL-1 on feelings of fatigue and behavior modulation, the synthesis of IL-1 from perivascular and meningeal macrophages during strenuous exercise has to be considered when approaching the complexity of exercise-induced fatigue. Changes in cerebral haemodynamics are not investigated in this article but should be subject of further studies about the regulation of exercise performance. In order to get deeper insights into the brain metabolism during prolonged strenuous exercise and its relation to neuroinflammation, the hormesis-like dose response of brain macrophage activation during exercise should be investigated in future studies.

As presented here, the majority of aspects concerning neuroimmune-neuroenergetic interactions in sports performance are not very well established and need to be evaluated in the future. Therefore, it is inevitable to improve interdisciplinary research in this field.

REFERENCES

1. Matthews, G., Hancock, P. A., & Desmond, P. A. The handbook of operator fatigue. CRC Press, 2018.
2. Noakes TD. Fatigue is a Brain-Derived Emotion that Regulates the Exercise Behavior to Ensure the Protection of Whole Body Homeostasis. Front Physiol 3: 82, 2012.
3. Kayser B. Exercise starts and ends in the brain. Eur J Appl Physiol 90: 411-419, 2003.
4.. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 9: 46-56, 2008.
5. Hart BL. Biological basis of the behavior of sick animals. Neurosci Biobehav Rev 12: 123-137, 1988.
6. Fehrenbach E, Schneider ME. Trauma-induced systemic inflammatory response versus exercise-induced immunomodulatory effects. Sports Med 36: 373-384, 2006.
7. Vargas NT, Marino F. A neuroinflammatory model for acute fatigue during exercise. Sports Med 44: 1479-1487, 2014.
8. Carmichael MD, Davis JM, Murphy EA, Carson JA, Van Rooijen N. Role of brain macrophages on IL-1beta and fatigue following eccentric exercise-induced muscle damage. Brain Behav Immun 24: 564-568, 2010.
9. Straub RH. Insulin resistance, selfish brain, and selfish immune system: an evolutionarily positively selected program used in chronic inflammatory diseases. Arthritis Res Ther 16 Suppl 2: S4, 2014.
10. Mason S. Lactate Shuttles in Neuroenergetics-Homeostasis, Allostasis and Beyond. Front Neurosci 11: 43, 2017.
11. Matsui T, Omuro H, Liu YF, Soya M, Shima T. Astrocytic glycogen-derived lactate fuels the brain during exhaustive exercise to maintain endurance capacity. Proc Natl Acad Sci U S A 114: 6358-6363, 2017.
12. Robertson CV, Marino FE. Prefrontal and motor cortex EEG responses and their relationship to ventilatory thresholds during exhaustive incremental exercise. Eur J Appl Physiol 115: 1939-1948, 2015.
13. Dworak M, Diel P, Voss S, Hollmann W, Strüder HK. Intense exercise increases adenosine concentrations in rat brain: implications for a homeostatic sleep drive. Neuroscience 150: 789-795, 2007.
14. Zheng X, Hasegawa H. Administration of caffeine inhibited adenosine receptor agonist-induced decreases in motor performance, thermoregulation, and brain neurotransmitter release in exercising rats. Pharmacol Biochem Behav 140: 82-89, 2016.
15. Hillion J, Canals M, Torvinen M, Casado V, Scott R. Coaggregation, cointernalization, and codesensitization of adenosine A2A receptors and dopamine D2 receptors. J Biol Chem 277: 18091-18097, 2002.
16. Amann M. Significance of Group III and IV muscle afferents for the endurance exercising human. Clin Exp Pharmacol Physiol 39: 831-835, 2012.
17. Hill AV, Lupton H. Muscular Exercise, Lactic Acid, and the Supply and Utilization of Oxygen. QJM os-16: 135-171, 1923.
18. Brooks GA. Cell-cell and intracellular lactate shuttles. J Physiol 587: 5591-5600, 2009.
19. Garcia-Alvarez M, Marik P, Bellomo R. Sepsis-associated hyperlactatemia. Crit Care 18: 503, 2014.
20. Kemppainen J, Aalto S, Fujimoto T, Kalliokoski KK, Långsjö J. High intensity exercise decreases global brain glucose uptake in humans. J Physiol 568: 323-332, 2005.
21. Schurr A. Lactate: the ultimate cerebral oxidative energy substrate? J Cereb Blood Flow Metab 26: 142-152, 2006.
22. Dienel GA. Brain lactate metabolism: the discoveries and the controversies. J Cereb Blood Flow Metab 32: 1107-1138, 2012.
23. Morales-Alamo D, Losa-Reyna J, Torres-Peralta R, Martin-Rincon M, Perez-Valera M. What limits performance during whole-body incremental exercise to exhaustion in humans? J Physiol 593: 4631-4648, 2015.
24. Newsholme E, Acworth, IN, Bellotti P, Benzi, G, Ljungqvist, A. Amino acids, brain neurotransmitters and a functional link between muscle and brain that is important in sustained exercise. In World Symposium on Doping in Sport : Florence, Centro Affari, 10th-12th May, 1987; book exhibition catalogue, S. 25-. Rom: International Athletic Foundation, 1988.
25. Cordeiro LM, Guimarães JB, Wanner SP, La Guardia RB, Miranda RM. Inhibition of tryptophan hydroxylase abolishes fatigue induced by central tryptophan in exercising rats. Scand J Med Sci Sports 24: 80-88, 2014.
26. Strasser B, Geiger D, Schauer M, Gatterer H, Burtscher M, Fuchs D. Effects of Exhaustive Aerobic Exercise on Tryptophan-Kynurenine Metabolism in Trained Athletes. PLoS ONE 11: e0153617, 2016.
27. Meeusen R, Watson P, Hasegawa H, Roelands B, Piacentini MF. Central fatigue: the serotonin hypothesis and beyond. Sports Med 36: 881-909, 2006.
28. Taylor JL, Amann M, Duchateau J, Meeusen R, Rice CL. Neural Contributions to Muscle Fatigue: From the Brain to the Muscle and Back Again. Med Sci Sports Exerc 48: 2294-2306, 2016.
29. Balthazar CH, Leite LH, Ribeiro RM, Soares DD, Coimbra CC. Effects of blockade of central dopamine D1 and D2 receptors on thermoregulation, metabolic rate and running performance. Pharmacol Rep 62: 54-61, 2010
30. Morris G, Berk M, Walder K, Maes M. Central pathways causing fatigue in neuro-inflammatory and autoimmune illnesses. BMC Med 13: 28, 2015.
31. Nakatomi Y, Mizuno K, Ishii A, Wada Y, Tanaka M. Neuroinflammation in Patients with Chronic Fatigue Syndrome/Myalgic Encephalomyelitis: An ^{11}C-(R)-PK11195 PET Study. J Nucl Med 55: 945-950, 2014.
32. Patrick Neary J, Roberts AD, Leavins N, Harrison MF, Croll JC, Sexsmith JR. Prefrontal cortex oxygenation during incremental exercise in chronic fatigue syndrome. Clin Physiol Funct Imaging 28: 364-372, 2008.
33. Smith LL. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? Med Sci Sports Exerc 32: 317-331, 2000.
34. Blankert JP. Neuroinflammation in burnout patients. In Neuroinflammation in burnout patients, Conference Proceedings, 2014.
35. Vargas NT, Marino F. A neuroinflammatory model for acute fatigue during exercise. Sports Med 44: 1479-1487, 2014.
36. Gleeson M. Interleukins and exercise. The Journal of physiology, 529 Pt 1(Pt 1), 1, 2000.
37. Robson-Ansley PJ, de Milander L, Collins, M, Noakes TD. Acute interleukin-6 administration impairs athletic performance in healthy, trained male runners. Can. J. Appl. Physiol. 29(4): 411-418, 2004.

38. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol, 2012.
39. Steensberg A, Febbraio MA, Osada T, Schjerling P, van Hall G. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. J Physiol 537: 633-639, 2001.
40. Febbraio MA, Hiscock N, Sacchetti M, Fischer CP, Pedersen BK. Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. Diabetes 53: 1643-1648, 2004.
41. Steensberg A, Fischer CP, Keller C, Møller K, Pedersen BK. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am J Physiol Endocrinol Metab 285: E433-E437, 2003.
42. Hill EE, Zack E, Battaglini C, Viru M, Viru A, Hackney AC. Exercise and circulating Cortisol levels: The intensity threshold effect. J Endocrinol Invest 31: 587-591, 2008.
43. Carmichael MD, Davis JM, Murphy EA, Brown AS, Carson JA. Recovery of running performance following muscle-damaging exercise: relationship to brain IL-1beta. Brain Behav Immun 19: 445-452, 2005.
44. Carmichael MD, Davis JM, Murphy EA, Brown AS, Carson JA. Role of brain IL-1beta on fatigue after exercise-induced muscle damage. Am J Physiol Regul Integr Comp Physiol 291: R1344-R1348, 2006.
45. Zendedel A, Habib P, Dang J, Lammerding L, Hoffmann S. Omega-3 polyunsaturated fatty acids ameliorate neuroinflammation and mitigate ischemic stroke damage through interactions with astrocytes and microglia. J Neuroimmunol 278: 200-211, 2015.
46. Shi Z, Ren H, Huang Z, Peng Y, He B. Fish Oil Prevents Lipopolysaccharide-Induced Depressive-Like Behavior by Inhibiting Neuroinflammation. Mol Neurobiol 54: 7327-7334, 2017.
47. Djordjevic B, Baralic I, Kotur-Stevuljevic J, Stefanovic A, Ivanisevic J. Effect of astaxanthin supplementation on muscle damage and oxidative stress markers in elite young soccer players. J Sports Med Phys Fitness 52: 382-392, 2012.
48. Bruunsgaard H, Galbo H, Halkjaer-Kristensen J, Johansen TL, MacLean DA, Pedersen BK. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. J Physiol 499 (Pt 3): 833-841, 1997.
49. Simpson RJ, Cosgrove C, Chee MM, McFarlin BK, Bartlett DB. Senescent phenotypes and telomere lengths of peripheral blood T-cells mobilized by acute exercise in humans. Exerc Immunol Rev 16: 40-55, 2010.
50. Krüger K, Lechtermann A, Fobker M, Völker K, Mooren FC. Exercise-induced redistribution of T lymphocytes is regulated by adrenergic mechanisms. Brain Behav Immun 22: 324-338, 2008.
51. Fisher-Wellman K, Bloomer RJ. Acute exercise and oxidative stress: a 30 year history. Dyn Med 8: 1, 2009.
52. Fogarty MC, Hughes CM, Burke G, Brown JC, Trinick TR. Exercise-induced lipid peroxidation: Implications for deoxyribonucleic acid damage and systemic free radical generation. Environ Mol Mutagen 52: 35-42, 2011
53. Ostrowski K, Rhode T, Asp S, Schjerling P, Pedersen B. Pro□and anti□inflammatory cytokine balance in strenuous exercise in humans, 1999.
54. Gleeson M. Immune function in sport and exercise. J Appl Physiol 103: 693-699, 2007.
55. Heck TG, Schöler CM, de Bittencourt PI. HSP70 expression: does it a novel fatigue signalling factor from immune system to the brain? Cell Biochem Funct 29: 215-226, 2011.
56. Bårdsen K, Nilsen MM, Kvaløy JT, Norheim KB, Jonsson G, Omdal R. Heat shock proteins and chronic fatigue in primary Sjögren's syndrome. Innate Immun 22: 162-167, 2016.
57. Marshall JC. The gut as a potential trigger of exercise-induced inflammatory responses. Can J Physiol Pharmacol 76: 479-484, 1998.
58. Pals KL, Chang RT, Ryan AJ, Gisolfi CV. Effect of running intensity on intestinal permeability. J Appl Physiol 82: 571-576, 1997.
59. Snipe RMJ, Khoo A, Kitic CM, Gibson PR, Costa RJS. The impact of exertional-heat stress on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile. Eur J Appl Physiol 118: 389-400, 2018.
60. Yeh YJ, Law LY, Lim CL. Gastrointestinal response and endotoxemia during intense exercise in hot and cool environments. Eur J Appl Physiol 113: 1575-1583, 2013.
61. Mutlu E, Keshavarzian A, Engen P, Forsyth CB, Sikaroodi M, Gillevet P. Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. Alcohol Clin Exp Res 33: 1836-1846, 2009.
62. Shing CM, Peake JM, Lim CL, Briskey D, Walsh NP. Effects of probiotics supplementation on gastrointestinal permeability, inflammation and exercise performance in the heat. Eur J Appl Physiol 114: 93-103, 2014.
63. Brock-Utne JG, Gaffin SL, Wells MT, Gathiram P, Sohar E. Endotoxaemia in exhausted runners after a long-distance race. S Afr Med J 73: 533-536, 1988.
64. Lambert GP. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. J Anim Sci 87: E101-E108, 2009.
65. Guo S, Al-Sadi R, Said HM, Ma TY. Lipopolysaccharide causes an increase in intestinal tight junction permeability in vitro and in vivo by inducing enterocyte membrane expression and localization of TLR-4 and CD14. Am J Pathol 182: 375-387, 2013.
66. Dokladny K, Zuhl MN, Moseley PL. Intestinal epithelial barrier function and tight junction proteins with heat and exercise. J Appl Physiol 120: 692-701, 2016.
67. Shimada A, Hasegawa-Ishii S. Histological Architecture Underlying Brain-Immune Cell-Cell Interactions and the Cerebral Response to Systemic Inflammation. Front Immunol 8: 17, 2017.
68. Erickson MA, Dohi K, Banks WA. Neuroinflammation: a common pathway in CNS diseases as mediated at the blood-brain barrier. Neuroimmunomodulation 19: 121-130, 2012.
69. Dantzer R, Heijnen CJ, Kavelaars A, Laye S, Capuron L. The neuroimmune basis of fatigue. Trends Neurosci 37: 39-46, 2014.
70. Bailey DM, Evans KA, McEneny J, Young IS, Hullin DA. Exercise-induced oxidative-nitrosative stress is associated with impaired dynamic cerebral autoregulation and blood-brain barrier leakage. Exp Physiol 96: 1196-1207, 2011.
71. Vitkovic L, Konsman JP, Bockaert J, Dantzer R, Homburger V, Jacque C. Cytokine signals propagate through the brain. Mol Psychiatry 5: 604, 2000.
72. Kapural M, Krizanac-Bengez Lj, Barnett G, Perl J, Masaryk T. Serum S-100beta as a possible marker of blood-brain barrier disruption. Brain Res 940: 102-104, 2002.

73. Koh SX, Lee JK. S100B as a marker for brain damage and blood-brain barrier disruption following exercise. Sports Med 44: 369-385, 2014.
74. Stålnacke BM, Tegner Y, Sojka P. Playing ice hockey and basketball increases serum levels of S-100B in elite players: a pilot study. Clin J Sport Med 13: 292-302, 2003.
75. Papa L, Ramia MM, Edwards D, Johnson BD, Slobounov SM. Systematic review of clinical studies examining biomarkers of brain injury in athletes after sports-related concussion. J Neurotrauma 32: 661-673, 2015.
76. Loane DJ, Kumar A. Microglia in the TBI brain: The good, the bad, and the dysregulated. Exp Neurol 275 Pt 3: 316-327, 2016.
77. Mollayeva T, Kendzerska T, Mollayeva S, Shapiro CM, Colantonio A, Cassidy JD. A systematic review of fatigue in patients with traumatic brain injury: the course, predictors and consequences. Neurosci Biobehav Rev 47: 684-716, 2014.
78. Ponsford JL, Ziino C, Parcell DL, Shekleton JA, Roper M. Fatigue and sleep disturbance following traumatic brain injury – their nature, causes, and potential treatments. J Head Trauma Rehabil 27: 224-233, 2012.
79. Erickson MA, Banks WA. Cytokine and chemokine responses in serum and brain after single and repeated injections of lipopolysaccharide: multiplex quantification with path analysis. Brain Behav Immun 25: 1637-1648, 2011.
80. Banks WA, Robinson SM. Minimal penetration of lipopolysaccharide across the murine blood-brain barrier. Brain Behav Immun 24: 102-109, 2010.
81. Banks WA, Gray AM, Erickson MA, Salameh TS, Damodarasamy M. Lipopolysaccharide-induced blood-brain barrier disruption: roles of cyclooxygenase, oxidative stress, neuroinflammation, and elements of the neurovascular unit. J Neuroinflammation 12: 223, 2015.
82. Quan N, Stern EL, Whiteside MB, Herkenham M. Induction of pro-inflammatory cytokine mRNAs in the brain after peripheral injection of subseptic doses of lipopolysaccharide in the rat. J Neuroimmunol 93: 72-80, 1999.
83. Black EAE, Cancelliere NM, Ferguson AV. Regulation of Nervous System Function by Circumventricular Organs, Neuroimmune Pharmacology. 25-37, 2017.
84. Vallières L, Rivest S. Regulation of the genes encoding interleukin-6, its receptor, and gp130 in the rat brain in response to the immune activator lipopolysaccharide and the proinflammatory cytokine interleukin-1beta. J Neurochem 69: 1668-1683, 1997.
85. Roth J, Harré EM, Rummel C, Gerstberger R, Hübschle T. Signaling the brain in systemic inflammation: role of sensory circumventricular organs. Front Biosci 9: 290-300, 2004.
86. Chakravarty S, Herkenham M. Toll-like receptor 4 on nonhematopoietic cells sustains CNS inflammation during endotoxemia, independent of systemic cytokines. J Neurosci 25: 1788-1796, 2005.
87. Nakano Y, Furube E, Morita S, Wanaka A, Nakashima T, Miyata S. Astrocytic TLR4 expression and LPS-induced nuclear translocation of STAT3 in the sensory circumventricular organs of adult mouse brain. J Neuroimmunol 278: 144-158, 2015.
88. Furube E, Morita M, Miyata S. Characterization of neural stem cells and their progeny in the sensory circumventricular organs of adult mouse. Cell Tissue Res 362: 347-365, 2015.
89. Thayer JF, Sternberg EM. Neural aspects of immunomodulation: focus on the vagus nerve. Brain Behav Immun 24: 1223-1228, 2010.
90. Berthoud H-R, Neuhuber WL. Functional and chemical anatomy of the afferent vagal system. Auton Neurosci 85: 1-17, 2000.
91. Dantzer R, Heijnen CJ, Kavelaars A, Laye S, Capuron L. The neuroimmune basis of fatigue. Trends Neurosci 37: 39-46, 2014.
92. de La Serre CB, de Lartigue G, Raybould HE. Chronic exposure to low dose bacterial lipopolysaccharide inhibits leptin signaling in vagal afferent neurons. Physiol Behav 139: 188-194, 2015.
93. Fleshner M, Goehler LE, Schwartz BA, McGorry M, Martin D. Thermogenic and corticosterone responses to intravenous cytokines (IL-1beta and TNF-alpha) are attenuated by subdiaphragmatic vagotomy. J Neuroimmunol 86: 134-141, 1998.
94. Lavoie JM. The contribution of afferent signals from the liver to metabolic regulation during exercise. Can J Physiol Pharmacol 80: 1035-1044, 2002.
95. Pereira BC, Lucas G, da Rocha AL, Pauli JR, Ropelle ER. Eccentric Exercise Leads to Glial Activation but not Apoptosis in Mice Spinal Cords. Int J Sports Med 36: 378-385, 2015.
96. Alvarez P, Levine JD, Green PG. Eccentric exercise induces chronic alterations in musculoskeletal nociception in the rat. Eur J Neurosci 32: 819-825, 2010.
97. Chiang CY, Sessle BJ, Dostrovsky JO. Role of astrocytes in pain. Neurochem Res 37: 2419-2431, 2012.
98. Chacur M, Lambertz D, Hoheisel U, Mense S. Role of spinal microglia in myositis-induced central sensitisation: an immunohistochemical and behavioural study in rats. Eur J Pain 13: 915-923, 2009.
99. Louati K, Berenbaum F. Fatigue in chronic inflammation - a link to pain pathways. Arthritis Res Ther 17: 254, 2015.
100. Maes M, Leunis JC. Normalization of leaky gut in chronic fatigue syndrome (CFS) is accompanied by a clinical improvement: effects of age, duration of illness and the translocation of LPS from gram-negative bacteria. Neuro Endocrinol Lett 29: 902-910, 2008.
101. Pal GD, Shaikh M, Forsyth CB, Ouyang B, Keshavarzian A, Shannon KM. Abnormal lipopolysaccharide binding protein as marker of gastrointestinal inflammation in Parkinson disease. Front Neurosci 9: 306, 2015.
102. Köhler CA, Maes M, Slyepchenko A, Berk M, Solmi M. The Gut-Brain Axis, Including the Microbiome, Leaky Gut and Bacterial Translocation: Mechanisms and Pathophysiological Role in Alzheimer's Disease. Curr Pharm Des 22: 6152-6166, 2016.
103. Berk M, Williams LJ, Jacka FN, O'Neil A, Pasco JA. So depression is an inflammatory disease, but where does the inflammation come from? BMC Med 11: 200, 2013.
104. Gárate I, Garcia-Bueno B, Madrigal JL, Caso JR, Alou L. Stress-induced neuroinflammation: role of the Toll-like receptor-4 pathway. Biol Psychiatry 73: 32-43, 2013.
105. Arakawa H, Blandino P, Deak T. Central infusion of interleukin-1 receptor antagonist blocks the reduction in social behavior produced by prior stressor exposure. Physiol Behav 98: 139-146, 2009.
106. Vollmer-Conna U, Fazou C, Cameron B, Li H, Brennan C. Production of pro-inflammatory cytokines correlates with the symptoms of acute sickness behaviour in humans. Psychol Med 34: 1289-1297, 2004.

107. Puri BK, Holmes J, Hamilton G. Eicosapentaenoic acid-rich essential fatty acid supplementation in chronic fatigue syndrome associated with symptom remission and structural brain changes. Int J Clin Pract 58: 297-299, 2004.
108. Lochmiller RL, Deerenberg C. Trade-offs in evolutionary immunology: just what is the cost of immunity? OIKOS 88: 87-99, 2000.
109. McEwen BS. Allostasis and allostatic load: implications for neuropsychopharmacology. Neuropsychopharmacology 22: 108-124, 2000.
110. Delmastro-Greenwood MM, Piganelli JD. Changing the energy of an immune response. Am J Clin Exp Immunol 2: 30-54, 2013.
111. Orihuela R, McPherson CA, Harry GJ. Microglial M1/M2 polarization and metabolic states. Br J Pharmacol 173: 649-665, 2016.
112. Mason S, van Furth AM, Mienie LJ, Engelke UF, Wevers RA. A hypothetical astrocyte-microglia lactate shuttle derived from a 1H NMR metabolomics analysis of cerebrospinal fluid from a cohort of South African children with tuberculous meningitis. Metabolomics 11: 822-837, 2015.
113. Matsui T, Soya S, Kawanaka K, Soya H. Brain Glycogen Decreases During Intense Exercise Without Hypoglycemia: The Possible Involvement of Serotonin. Neurochem Res 40: 1333-1340, 2015.
114. Simpson RJ, Florida-James GD, Cosgrove C, Whyte GP, Macrae S. High-intensity exercise elicits the mobilization of senescent T lymphocytes into the peripheral blood compartment in human subjects. J Appl Physiol 103: 396-401, 2007.
115. Gleeson M. Immune function in sport and exercise. Edinburgh; New York: Churchill Livingstone Elsevier: British Association of Sport and Exercise Sciences, 2006.
116. Dimeloe S, Burgener AV, Grählert J, Hess C. T-cell metabolism governing activation, proliferation and differentiation; a modular view. Immunology 150: 35-44, 2017.
117. Brown FF, Bigley AB, Sherry C, Neal CM, Witard OC. Training status and sex influence on senescent T-lymphocyte redistribution in response to acute maximal exercise. Brain Behav Immun 39: 152-159, 2014.
118. Spielmann G, McFarlin BK, O'Connor DP, Smith PJ, Pircher H, Simpson RJ. Aerobic fitness is associated with lower proportions of senescent blood T-cells in man. Brain Behav Immun 25: 1521-1529, 2011.
119. Bours MJ, Swennen EL, Di Virgilio F, Cronstein BN, Dagnelie PC. Adenosine 5'-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. Pharmacol Ther 112: 358-404, 2006.
120. Bajgar A, Kucerova K, Jonatova L, Tomcala A, Schneedorferova I. Extracellular adenosine mediates a systemic metabolic switch during immune response. PLoS Biol 13: e1002135, 2015.
121. Dolezal, T. Adenosine: A selfish-immunity signal? Oncotarget 6: 32307-32308, 2015
122. Boison D, Chen JF, Fredholm BB. Adenosine signaling and function in glial cells. Cell Death Differ 17: 1071-1082, 2010.
123. Cunha RA. Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors. Neurochem Int 38: 107-125, 2001.
124. Choi J, Tanaka K, Cao Y, Qi Y, Qiu J. Identification of a Plant Receptor for Extracellular ATP. Science 343: 290-294, 2014.
125. Gaikwad S, Patel D, Agrawal-Rajput R. CD40 Negatively Regulates ATP-TLR4-Activated Inflammasome in Microglia. Cell Mol Neurobiol 37: 351-359, 2017.
126. Beamer E, Gölöncsér F, Horváth G, Bekő K, Otrokocsi L. Purinergic mechanisms in neuroinflammation: An update from molecules to behavior. Neuropharmacology 104: 94-104, 2016.
127. Sperlágh B, Baranyi M, Haskó G, Vizi ES. Potent effect of interleukin-1 beta to evoke ATP and adenosine release from rat hippocampal slices. J Neuroimmunol 151: 33-39, 2004.
128. Saura J, Angulo E, Ejarque A, Casadó V, Tusell JM. Adenosine A2A receptor stimulation potentiates nitric oxide release by activated microglia. J Neurochem 95: 919-929, 2005.
129. Villalta SA, Nguyen HX, Deng B, Gotoh T, Tidball JG. Shifts in macrophage phenotypes and macrophage competition for arginine metabolism affect the severity of muscle pathology in muscular dystrophy. Hum Mol Genet 18: 482-496, 2009.
130. Shieh CH, Heinrich A, Serchov T, van Calker D, Biber K. P2X7-dependent, but differentially regulated release of IL-6, CCL2, and TNF-α in cultured mouse microglia. Glia 62: 592-607, 2014.
131. Pocock JM, Kettenmann H. Neurotransmitter receptors on microglia. Trends Neurosci 30: 527-535, 2007.
132. Ferre S, Euler GV, Johansson B, Fredholm BB, Fuxe K. Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. Proc Natl Acad Sci U S A 88: 7238-7241, 1991.
133. Beggiato S, Tomasini MC, Borelli AC, Borroto-Escuela DO, Fuxe K. Functional role of striatal A2A, D2, and mGlu5 receptor interactions in regulating striatopallidal GABA neuronal transmission. J Neurochem 138: 254-264, 2016.
134. Correa M, Pardo M, Bayarri P, López-Cruz L, San Miguel N. Choosing voluntary exercise over sucrose consumption depends upon dopamine transmission: effects of haloperidol in wild type and adenosine A□AKO mice. Psychopharmacology 233: 393-404, 2016.
135. Zheng X, Hasegawa H. Administration of caffeine inhibited adenosine receptor agonist-induced decreases in motor performance, thermoregulation, and brain neurotransmitter release in exercising rats. Pharmacol Biochem Behav 140: 82-89, 2016.
136. Zheng X, Takatsu S, Wang H, Hasegawa H. Acute intraperitoneal injection of caffeine improves endurance exercise performance in association with increasing brain dopamine release during exercise. Pharmacol Biochem Behav 122: 136-143, 2014.
137. Roelands B, Buyse L, Pauwels F, Delbeke F, Deventer K, Meeusen R. No effect of caffeine on exercise performance in high ambient temperature. Eur J Appl Physiol 111: 3089-3095, 2011.
138. Krüger K, Mooren FC. Exercise-induced leukocyte apoptosis. Exerc Immunol Rev 20: 117-134, 2014.
139. Wang JS, Huang YH. Effects of exercise intensity on lymphocyte apoptosis induced by oxidative stress in men. Eur J Appl Physiol 95: 290-297, 2005.
140. Turner JE, Bosch JA, Drayson MT, Aldred S. Assessment of oxidative stress in lymphocytes with exercise. J Appl Physiol 111: 206-211, 2011.
141. Rock KL, Kono H. The inflammatory response to cell death. Annu Rev Pathol 3: 99-126, 2008.

142. Goehler LE, Gaykema RP, Nguyen KT, Lee JE, Tilders FJ. Interleukin-1beta in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems? J Neurosci 19: 2799-2806, 1999.
143. Margeli A, Skenderi K, Tsironi M, Hantzi E, Matalas AL. Dramatic elevations of interleukin-6 and acute-phase reactants in athletes participating in the ultradistance foot race spartathlon: severe systemic inflammation and lipid and lipoprotein changes in protracted exercise. J Clin Endocrinol Metab 90: 3914-3918, 2005.
144. Biber K, Neumann H, Inoue K, Boddeke HW. Neuronal 'On' and 'Off' signals control microglia. Trends Neurosci 30: 596-602, 2007.
145. Haskó G, Pacher P, Vizi ES, Illes P. Adenosine receptor signaling in the brain immune system. Trends Pharmacol Sci 26: 511-516, 2005.
146. Carman AJ, Mills JH, Krenz A, Kim DG, Bynoe MS. Adenosine receptor signaling modulates permeability of the blood-brain barrier. J Neurosci 31: 13272-13280, 2011.
147. Calabrese EJ. Hormesis: principles and applications. Homeopathy 104: 69-82, 2015.
148. Radak Z, Ishihara K, Tekus E, Varga C, Posa A. Exercise, oxidants, and antioxidants change the shape of the bell-shaped hormesis curve. Redox Biol 12: 285-290, 2017.
149. Gimeno-Bayón J, López-López A, Rodríguez MJ, Mahy N. Glucose pathways adaptation supports acquisition of activated microglia phenotype. J Neurosci Res 92: 723-731, 2014.
150. Moreira TJ, Pierre K, Maekawa F, Repond C, Cebere A. Enhanced cerebral expression of MCT1 and MCT2 in a rat ischemia model occurs in activated microglial cells. J Cereb Blood Flow Metab 29: 1273-1283, 2009.
151. Robertson CV, Marino FE. Cerebral responses to exercise and the influence of heat stress in human fatigue. J Therm Biol 63: 10-15, 2017.
152. Juffermans NP, Verbon A, van Deventer SJ, Buurman WA, van Deutekom H. Serum concentrations of lipopolysaccharide activity-modulating proteins during tuberculosis. J Infect Dis 178: 1839-1842, 1998.
153. Peterson PK, Hu S, Sheng WS, Kravitz FH, Molitor TW. Thalidomide Inhibits Tumor Necrosis Factor-α Production by Lipopolysaccharide- and Lipoarabinomannan-Stimulated Human Microglial Cells. J Infect Dis 172: 1137-1140, 1995.
154. Wheeler DS, Chase MA, Senft AP, Poynter SE, Wong HR, Page K. Extracellular Hsp72, an endogenous DAMP, is released by virally infected airway epithelial cells and activates neutrophils via Toll-like receptor (TLR)-4. Respir Res 10: 31, 2009.
155. Vabulas RM, Ahmad-Nejad P, Ghose S, Kirschning CJ, Issels RD, Wagner H. HSP70 as endogenous stimulus of the Toll/interleukin-1 receptor signal pathway. J Biol Chem 277: 15107-15112, 2002.
156. Dalsgaard MK, Quistorff B, Danielsen ER, Selmer C, Vogelsang T, Secher NH. A reduced cerebral metabolic ratio in exercise reflects metabolism and not accumulation of lactate within the human brain. J Physiol 554: 571-578, 2004.
157. Mason S, Reinecke CJ, Kulik W, van Cruchten A, Solomons R, van Furth AM. Cerebrospinal fluid in tuberculous meningitis exhibits only the L-enantiomer of lactic acid. BMC Infect Dis 16: 251, 2016.
158. Hanff TC, Furst SJ, Minor TR. Biochemical and anatomical substrates of depression and sickness behavior. Isr J Psychiatry Relat Sci 47: 64-71, 2010.
159. Del Cerro, S., Borrell J. Interleukin-1 Affects the Behavioral Despair Response in Rats by An Indirect Mechanism Which Requires Endogenous CRF. Brain Research, 528: 162-164, 1990.
160. Holst SC, Landolt H-P. Sleep Homeostasis, Metabolism, and Adenosine. Current Sleep Medicine Reports 1: 27-37, 2015.
161. Arrigoni E, Chamberlin NL, Saper CB, McCarley RW. Adenosine inhibits basal forebrain cholinergic and noncholinergic neurons in vitro. Neuroscience 140: 403-413, 2006.
162. Basheer R, Porkka-Heiskanen T, Strecker RE, Thakkar MM, McCarley RW. Adenosine as a biological signal mediating sleepiness following prolonged wakefulness. Biol Signals Recept 9: 319-327, 2000.
163. Shao W, Zhang SZ, Tang M, Zhang XH, Zhou Z. Suppression of neuroinflammation by astrocytic dopamine D2 receptors via αB-crystallin. Nature 494: 90-94, 2013.
164. Bjerring PN, Dale N, Larsen FS. Acute hyperammonemia and systemic inflammation is associated with increased extracellular brain adenosine in rats: a biosensor study. Neurochem Res 40: 258-264, 2015.
165. Wilkinson DJ, Smeeton NJ, Watt PW. Ammonia metabolism, the brain and fatigue; revisiting the link. Prog Neurobiol 91: 200-219, 2010.
166. Robertson CV, Marino FE. A role for the prefrontal cortex in exercise tolerance and termination. J Appl Physiol 120: 464-466, 2016.
167. St Clair Gibson A, De Koning JJ, Thompson KG, Roberts WO, Micklewright D. Crawling to the finish line: why do endurance runners collapse? Implications for understanding of mechanisms underlying pacing and fatigue. Sports Med 43: 413-424, 2013.

Inflammatory features of obesity and smoke exposure and the immunologic effects of exercise

Helena Angelica Pereira Batatinha[1], Jose Cesar Rosa Neto[1], Karsten Krüger[2]

[1] Immunometabolism Research Group, Department of Cell Biology and Development, Institute of Biomedical Sciences, University of São Paulo, São Paulo, SP, Brazil.
[2] Department of Exercise and Health, Institute of Sports Science, Leibniz University of Hannover, Hannover, Germany.

ABSTRACT

Many lifestyle-related diseases, such as obesity and cigarette smoke-induced pulmonary morbidities, are associated with chronic systemic inflammation, which has been shown to contribute to the disease initiation and progression, and also for co-morbidities of these diseases. While the source of inflammation in obese subjects is suggested to be mainly the visceral adipose tissue, smoke-induced inflammation originates in the pulmonary system. Here, chronic cigarette smoking induces oxidative stress, resulting in severe cellular damage. During obesity, metabolic stress pathways in adipocytes induce inflammatory cascades which are also accompanied by fibrotic processes and insulin resistance. In both diseases, local inflammatory signals induce progressive immune cell infiltration, release of cytokines and a subsequent spill-over of inflammation to the systemic circulation. Exercise training represents an effective therapeutic and immune regulating strategy for both obese patients, as well as for patients with smoke induced pulmonary inflammation. While the immune-regulating impact of exercise might primarily depend on the disease state, patients with pulmonary inflammation seem to be less responsive to exercise therapy. The current review tries to identify similarities and differences between inflammatory processes, and the consequences for the immunoregulatory effects of exercise as a therapeutic agent.

Keywords:immune system, cytokines, physical activity, adipose tissue, cigarette smoking

Address for correspondence:
Helena Batatinha, Department of Cell and Developmental Biology, University of São Paulo. 1524,
Prof Lineu Prestes Av. Sao Paulo, SP, Brazil,05508-000.
+55 155 3091 0883
e-Mail: batatinha.helena@usp.br

1. INTRODUCTION

Many lifestyle-related diseases—such as diabetes type II, coronary heart disease (CHD), cancer, obesity or chronic obstructive pulmonary disease (COPD)—are associated with chronic systemic inflammation. Beyond mutual association, systemic inflammatory processes have been shown to contribute to the initiation and progression of these diseases (1), and are suggested to be an important reason for their shared comorbidities. Most of these diseases share some similarities in their inflammatory genesis, while other related processes are distinctly different. A combination of two or more of these diseases has been shown to further aggravate morbidity and mortality (2). Exercise is known to be an effective treatment for most of these diseases, at least partly due to its immune-regulating properties (3).

In this regard, it has been shown that patients with specific diseases, such as chronic obstructive pulmonary disease (COPD), are less responsive to exercise therapy compared with other diseases, such as obesity (4). However, until now, there are few data available to prescribe different exercise guidelines for specific disease conditions characterized by a dysregulated immune system. In order to better understand the inflammatory pathophysiological genesis of lifestyle-related diseases, the current review tries to compare the inflammatory processes at work in two examples of highly prevalent life style-related diseases: obesity and long-term cigarette smoking. Knowing the specific cellular and molecular mechanisms of inflammation initiation and progression will enable more precise and safe application of exercise therapy for each disease and individual case.

2. SYSTEMIC LOW-GRADE INFLAMMATION DURING OBESITY

Obesity is a major global health issue. The World Health Organization (5) update (2016) showed that around 39% of people over 18 years were overweight (BMI >25 kg/m^2), while 13% were obese (BMI >30 kg/m^2). Obesity increases the risk of mortality (6,7) through its strong association with other comorbidities, such as Type 2 diabetes mellitus (T2DM), cardiovascular diseases, insulin resistance, non-

alcoholic fat liver disease (NAFLD), osteoarthritis, and autoimmune diseases (7,8). In addition to their personal burden, these disease have deep economic and social impacts on Western society (9).

Obesity development depends on different factors, including genetic, epigenetic, physiological, and environmental factors (10). The link between obesity and these associated comorbidities is, at least partly, the chronic low-grade inflammation that represents one of the hallmarks of obesity (11). Excepting a small subgroup of apparently metabolically healthy obese individuals, most obese patients develop chronic low-grade inflammation. This leads to metabolic and physiological perturbations that ultimately result in immunometabolic alterations in organs and tissues such as liver, brain, skeletal and cardiac muscle, blood vessels, lung, kidney, gut, and immune systems (12,13).

2.1. Sources of inflammation

The low-grade systemic inflammation in obese subjects originates in visceral adipose tissue, caused by the hypertrophy of adipocytes and cellular stress signals. These signals dysregulate adipokine production, and increase the release of pro-inflammatory adipokines, cytokines, phase acute proteins, and chemokines (14) (Figure 1).

Over the past decade, adipose tissue has been studied as an active metabolic and endocrine organ that mainly regulates energy expenditure and glucose homeostasis through adipokine production. It is a complex organ, composed of adipocytes, fibroblasts, endothelial cells, and a wide-range of immune cells, giving it an important immune-modulator function (15).

Adiponectin and several other cytokines are released by lean adipose tissue. These adipokines play a role in maintaining an anti-inflammatory status. Adiponectin induces interleukin (IL)-10 and IL-1ra in macrophages, together with polarization of alternatively-activated macrophages (M2) (16). Moreover, the adipokines mitigate the production of the pro-inflammatory cytokines in adipocytes and macrophages stimulated by lipopolysaccharide (LPS) (17,18). Furthermore, lean adipocytes release anti-inflammatory cytokines IL-4, IL-5, IL-10, IL-1ra, IL-13, and IL-33, recruiting and polarizing infiltrated immune cells to an anti-inflammatory phenotype (19,20).

The increase in energy intake (usually the result of a high-fat diet) and decrease in energy expenditure (usually the result of a sedentary lifestyle) leads to a positive energy balance and fat accumulation in the adipocytes, which is caused by the dysregulation of the lipolysis/lipogenesis ratio (21). This hypertrophy of the adipose tissue plays a key role in the development of obesity-induced local inflammation (10).

Hypertrophic adipocytes activate stress-sensing pathways in response to metabolic and oxidative stress, increasing the phosphorylation of intracellular kinases as p38MAPK, Jun N-terminal kinase (JNK), and inhibitory-κB kinase (IKK) (22). Adipocyte size and p38MAPK, as well as JNK phosphorylation in the visceral fat of obese humans have been positively correlated with adipose tissue inflammation and whole-body insulin resistance (23,24). JNK and IKK phosphorylation activates activator protein-1 (AP-1) and nuclear factor kappa B (NF-κB) transcription factors (25). This increases the gene expression of many pro-inflammatory cytokines. The NLRP3 inflammasome also plays a key role in adipocyte-hypertro-

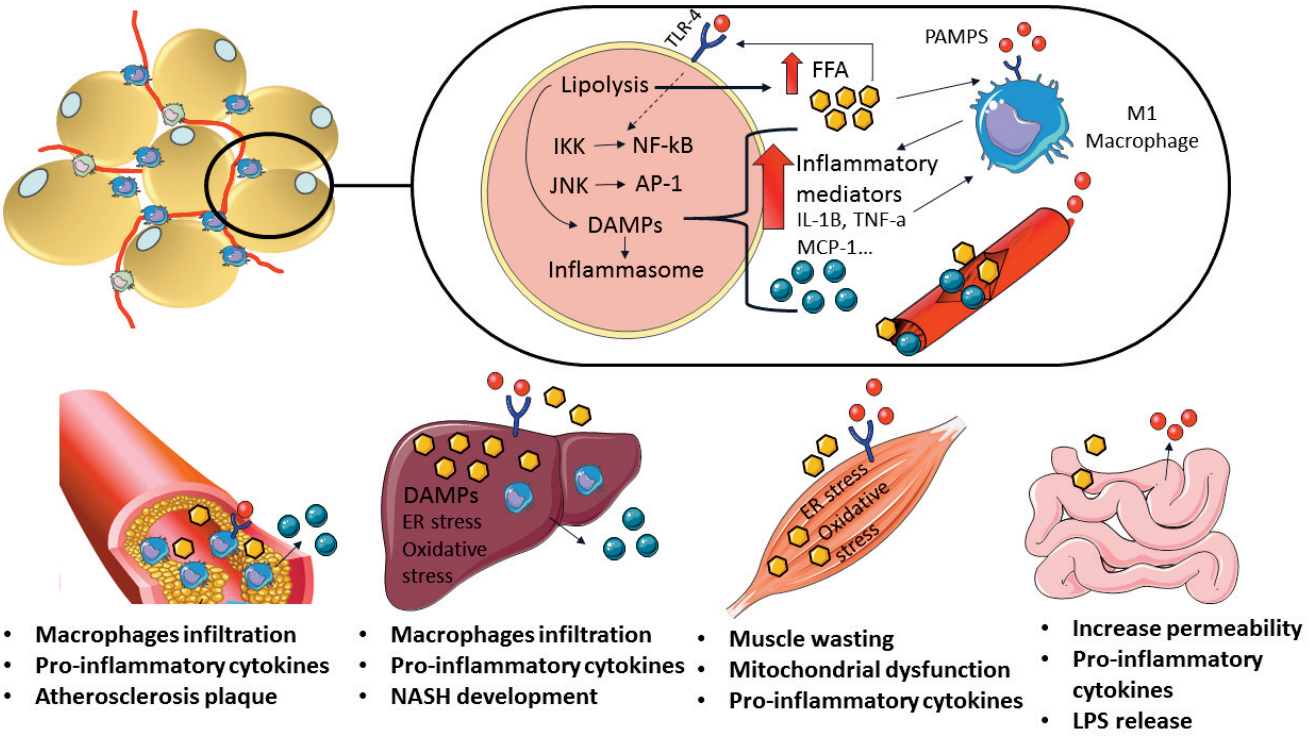


Figure 1: Overview about how obesity induces local and systemic inflammation and its manifestations in blood, liver, muscle, and gut. (PAMPs = pathogen-associated molecular patterns, FFAs= free fatty acids, IKK = inhibitor of κ kinase; JNK: c-Jun N-terminal kinase; TLR4 = Toll-like receptor-4, AP-1: activator protein-1, NF-kB= nuclear factor kappa B, LPS= lipopolysaccharide)

phy-induced inflammation. It is activated in response to intracellular danger-associated molecular patterns (DAMPs), such as adenosine triphosphate (ATP), uric acid, an accumulation of palmitate, and advanced glycation end-products, which are all known to be increased in obesity (26) (Figure 1).

After this signal processing, fat adipocytes increase the production of pro-inflammatory cytokines, as monocyte chemoattractant protein (MCP)-1, IL-1β, IL-6, and tumor necrosis factor (TNF)-α. This is accompanied by a reduction of adiponectin and other anti-inflammatory cytokines. Thus, an imbalance of the inflammatory mediators results that is tilted toward a pro-inflammatory response, recruiting and polarizing immune cells to a pro-inflammatory phenotype.

2.2. Local and systemic effects

Adipocytes express surface stress ligands that are recognized by natural killer (NK) cells. The activation of NK cells leads to higher production of interferon-gamma (IFN-γ), which is essential for polarizing macrophages towards a pro-inflammatory phenotype (M1) (18). Macrophages represent up to 60% of all immune cells in the vascular stromal portion of adipose tissue (27). Adipose tissue macrophages are the major players in pro-inflammatory cytokine production in obese adipose tissue (28,29). It was previously postulated that all adipose tissue macrophages were driven by circulating monocytes, which infiltrate and differentiate to macrophages. However, in 2014, Amano and collaborators showed that macrophages from visceral adipose tissue were able to proliferate during obesity, and monocyte depletion had no significant impact on the number of adipose tissue macrophages (30). They also elucidated that macrophage proliferation was MCP-1 dependent. Interestingly, these adipose tissue macrophages express the molecule CD11c, which is mainly known to be expressed by dendritic cells. Wouters and his group (31) found a positive correlation between CD11c+ adipose tissue macrophages from visceral depots and classical monocytes numbers in the circulation, suggesting that the classic macrophages originating from monocytes may also play a role in this obesity-induced adipose tissue macrophage phenotype.

CD11c+ adipose tissue macrophage cells play a key role in the development of insulin resistance. Depleting these cells from obese mice can restore insulin sensitivity and decrease local and systemic pro-inflammatory cytokine production (32). Moreover, recent findings indicate that numbers of CD11c+ adipose tissue macrophages from visceral depots are directly correlated with non-alcoholic steatohepatitis (NASH) development. These cells appear to induce macrophage and neutrophil accumulation in the liver (33).

CD11c+ adipose tissue macrophages accumulate in visceral adipose tissue during obesity, forming crown-like structures (CLS) and producing great amounts of pro-inflammatory cytokines. They are therefore important contributors to the metabolic syndrome development. Toll-like receptor 4 (TLR-4) has been proposed to play a key role in this process. TLRs recognize pathogen-associated molecular patterns (PAMPs) and DAMPs, and induce a downstream signaling to activate NF-κB (34), and then induce pro-inflammatory cytokine transcription. Furthermore, TLR-4 seems to have a direct impact on obesity-induced accumulation of CD11c+ adipose tissue macrophages and adipose tissue pathology. Knockout mice for TLR-4 showed low activation of CD11c+ adipose tissue macrophages after a high-fat diet, followed by a decrease in adipose tissue fibrosis (35). Moreover, wild-type mice injected with bone marrow from TLR-4 -/- mice presented low adiposity, improved glucose tolerance, and reduced accumulation of CD11c+ adipose tissue macrophages in visceral adipose tissue (36). This suggests that the expression of TLR-4 in myeloid cells is necessary for monocyte recruitment and CD11c+ adipose tissue macrophage accumulation. In addition, both TLR-4 and MyD88 full body knockout mice showed the same adipocyte hypertrophy as wild-type animals, however they exhibited much lower CD11c+ adipose tissue macrophage percentages, crown-like structure formations and fibrosis (36), indicating that the TLR-4/MyD88 pathway is necessary for obesity-induced adipose tissue pathology.

During the past few years, it was established that saturated fatty acids (especially palmitate) could activate TLR-4 and then contribute to obesity-induced metabolic syndrome. Cameron Griffin and collaborators (2018) found that obese TLR-4 and MyD88 knockout mice generate fewer myeloid colonies compared to wild-type mice (36). These animals also failed to develop a palmitate or LPS-induced myeloid colony formation. Increased numbers of myeloid progenitors are observed in high fat diet-induced obesity and inflammatory macrophages mostly originate from these progenitors (37). In addition, TLR-4 expression in myeloid cells are necessary for insulin resistance and adipose tissue inflammation during obesity (38). These findings suggest that TLR-4 is required for diet-induced inflammation.

Other work has shown that TLR-4 knockout mice are protected from saturated fatty acid-induced inflammation (39,40). In this regard, Lancaster's group (41) identified that saturated fatty acids are not TLR-4 agonists, they are not able to form a TLR-4/MD-2 dimer and induce its downstream signaling. They also found a protection from saturated fatty acid-induced inflammation in those mice who lack TLR-4 (41). Finally, they discovered that saturated fatty acids cannot bind directly to TLR-4, but they play a secondary role in activating inflammatory signaling after an initial TLR4-dependent priming signal.

One of the most effective ways to activate TLR-4 signaling is through LPS stimulation. LPS is present at the membrane of gram-negative bacteria and is increased in the circulation during obesity. Intestinal permeability plays an important role in obesity-induced endotoxaemia. Many studies have reported a positive correlation between dietary fat consumption and plasma LPS concentration (42). Moreover, a recent investigation with non-obese and obese patients revealed that obese people had higher levels of markers for altered gastrointestinal barrier function in the serum, and showed a positive correlation between intestinal permeability and inflammatory markers (43). In addition, when the jejunum samples were challenge with lipids, both obese and non-obese samples presented increased intestinal permeability. However, samples obtained from the obese group had a 2-fold higher increase in permeability than the non-obese group, and took much longer to

restore the barrier function. Putting this all together, these data demonstrate that acute fat ingestion directly influences intestinal permeability, and chronic fat consumption may play a role in increasing intestinal and systemic inflammation (43).

2.3. Effect of obesity on other organs and tissues

Free fatty acids are toxic for several organs. The first step in the metabolism of lipids after digestion and absorption is regulated by the liver. Thus, nonalcoholic fatty liver disease (NAFLD) has reached alarming proportion in contemporary society (44). The trigger of NAFLD is the accumulation of triacylglycerol in hepatic parenchyma due to an imbalance between fatty oxidation and fatty storage (45). Interestingly, these two opposite pathways are regulated by the family of peroxisome proliferator-activated receptors (PPARs) (46). NAFLD can progress to NASH, cirrhosis, and hepatocellular carcinoma. This progression is dependent on hepatic inflammation, which is caused by Kupffer cells and an enhanced inflammatory response of hepatocytes (47). Furthermore, Ogawa et al (2018) showed that the increase in gut-derived endotoxin is necessary for the progression of NAFLD by inducing fibrosis and immune cells recruitment (48).

Excess fatty acid accumulation in hepatocytes leads to intracellular stress, (oxidative and endoplasmatic reticulum (ER) stress) inducing cell apoptosis (49). This internal signaling, associated with extracellular-induced TLR-4 activation by LPS, leads to a chronic and harmful inflammatory signal (50) (Figure 1). In NAFLD, the classical pro-inflammatory cytokines are increased (TNF-α, IL-6 and IL-1β), with an elevation of monocyte infiltration in the liver and macrophage differentiation to the M1 subset (i.e., higher expressions of CD11c, CD86) (51). The progression of NAFLD to NASH is characterized by the exacerbation of chronic inflammation and fibrosis, increasing fatty acid synthase (FAS), death receptor 5 (TRAILR-2), and TNF receptors, and a consequent increase in cytotoxic T lymphocytes (51).

In endothelial cells, higher levels of circulating free fatty acids, especially palmitate, induce a decrease in nitric oxide production, and increase the inflammatory response stimulated by LPS (52). The stressed endothelial cells increase the synthesis of integrins and selectins, thereby promoting immune cell infiltration in the vascular wall (53). Thus, monocytes and lymphocytes that have infiltrated into the vascular wall potentiate the inflammatory response, releasing pro-inflammatory cytokines (IL-1, TNF and IL-6) and chemokines (RANTES, IL-8 and MCP-1). The atherosclerotic plaque formation occurs when monocytes-derived macrophages phagocytize the lipid particles and induce foam cells (54) (Figure 1).

Skeletal muscle is a very important tissue for the maintenance of metabolic homeostasis. Elevated consumption of high-fat foods also induces lipid storage in the muscle, which increases diacylglycerol and ceramide accumulation inside myocytes (55). Both responses are related to increased cellular stress, observed by ER stress, together with enhanced unfolded protein response and mitochondria dysfunction, in turn causing oxidative stress (56). Additionally, the PAMPs and DAMPs that arise from lipid overload in obesity activate the TLR-4 pathway, triggering MAPK and NF-κB pathways (57). Pro-inflammatory cytokines and chemokines (MCP-1, IL-1β, IL-6 and TNF-α) are produced and released into circulation, leading to autocrine, paracrine, and endocrine effects (58) (Figure 1).

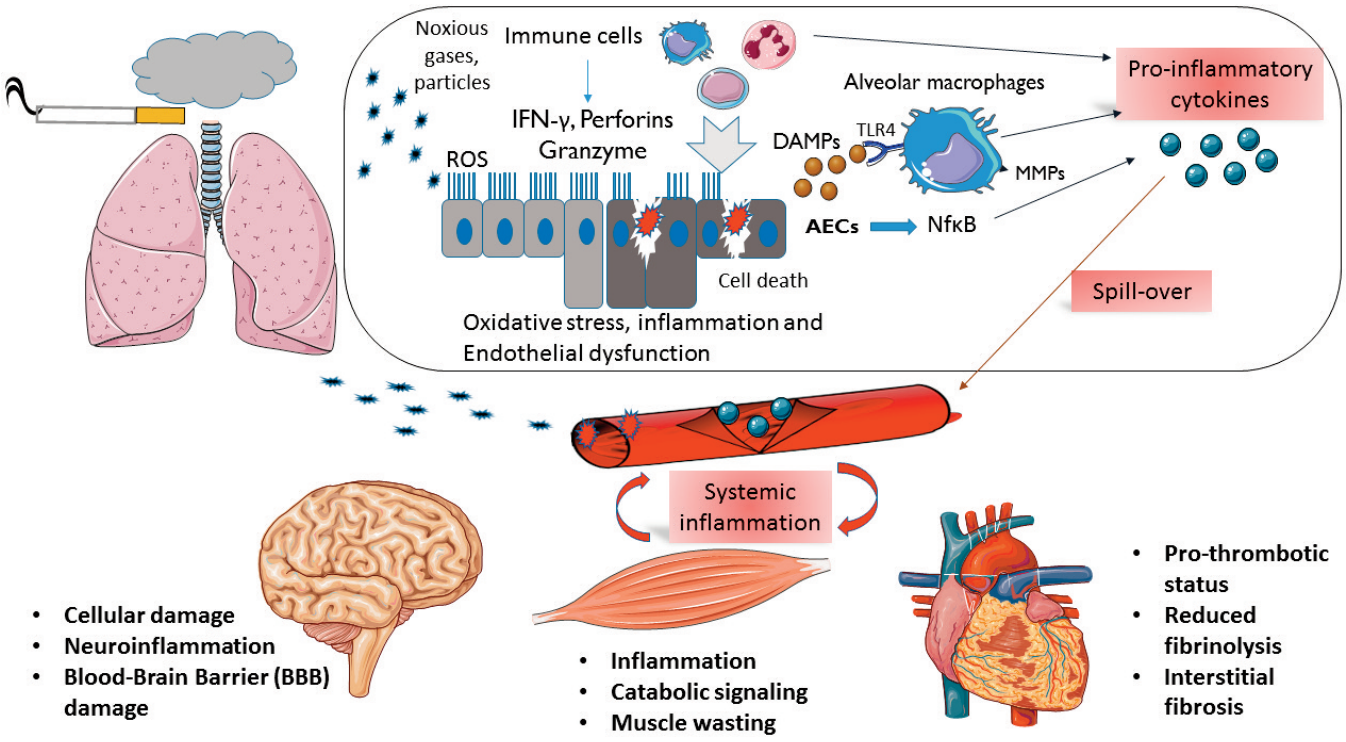
3. CIGARETTE SMOKE AS A BURDEN OF INFLAMMATION AND DISEASE

Tobacco smoking is a significant cause of morbidity and mortality, with approximately 5 million deaths caused by direct tobacco use and more than 600,000 deaths due to passive smoking worldwide every year. While in many countries intensified tobacco control efforts have resulted in a reduced prevalence of smoking, in other countries the number of smokers is steadily increasing. Cigarette smoking represents an important risk factor for cardiac infarction, stroke, and is the central risk factor for the development of bronchialcarcinoma, COPD, and smoking associated lung fibrosis. Smoke associated diseases, such as COPD, are today classified as systemic inflammatory diseases because sustained inflammatory processes seem to be main drivers of co-morbidities, such as muscle wasting, vascular diseases, heart diseases, and stroke (59,60).

3.1. Sources and origins of inflammation

Cigarette smoke contains a multitude of immunomodulatory chemicals and gases, making it complex to discern a clear origin of the associated inflammation. However, it is suggested that the repeated contact of airway cells with specific noxious gases and particles leads to repetitive stress signals and inflammatory insults that are followed in the long-term by a chronic and progressive activation of the immune system (61). Accordingly, inflammatory processes originate in the pulmonary system, where the regular contact with toxic substances disturb the barrier function of the respiratory epithelium. Studies that have analyzed the bronchoalveolar lavage fluid (BAL) or and breath condensate of acute or chronic smokers proved that oxidative stress is an important factor for tissue damage. This is indicated by an accumulation of products of lipid peroxidation and extracellular matrix proteins (62,63). Airway epithelial cells, which represent a first line of defense against inhaled toxicants, are an important inducer of these inflammatory processes. In smokers, airway epithelial cells show characteristics of cellular damage, followed by the release of DAMPs. These molecules act in a paracrine fashion by activating pattern recognition receptors, such as TLR4, expressed by airway epithelial cells and other cells in the pulmonary system. In addition, the inhalation of smoke particles might also directly activate pattern recognition receptors (64). Consequently, TLR4 expression is significantly upregulated on airway cells of smokers (Figure 2).

In response to TLR4 activation, a role for heat shock protein 70 (HSP70), a known TLR4 agonist, has been shown, which is induced in airways upon smoke exposure followed by the activation of the innate immune system through TLR4/MyD88. Subsequently, NF-κB is translocated into the nucleus, inducing the secretion of a variety of pro-inflammatory cytokines. Cigarette smoke exposure has also been shown to induce ER stress in airway epithelial cells and



Figures 2: Illustration about cigarette-induced induction of oxidative stress and inflammation in airway epithelial cells (AECs) and the characteristics of extrapulmonary manifestations of inflammatory signals from the pulmonary system to brain, muscle, and cardiac tissue (TLR4 = Toll-like receptor-4, DAMPs= damage-associated molecular patterns, MMP=matrix metalloproteases, ROS= reactive oxygen species).

mouse lungs, which is suggested to amplify the inflammatory processes (65). Similarly, excessive activation of the inflammasome has also been shown to play a critical role in the innate immune response against cigarette smoke-induced noxious stimuli in the lung tissue (66). Consequently, increased levels of various cytokines (such as IL-1β, IL-6, IL-8, MCP-1), macrophage inflammatory protein (MIP) 1α, regulated on activation normal T-cell expressed and secreted (RANTES), TNF-α, IL-12(p40), and IL-17 can be found in the BAL of long-term smokers (67). In addition, extracellular signal-regulated kinase signaling and increased p38MAPK in airway epithelial cells induces the expression of a variety of metalloproteases (MMPs), such as MMP9 and MMP12, and surfactant protein D, which can be also found in higher quantities in the lungs of long-term smokers (68,69) (Figure 2).

Cell damage, DAMP release, and the expression of chemokines are all triggers of immune cell invasion into the pulmonary system. Consequently, tissue resident or circulating macrophages, neutrophils, dendritic cells, and lymphocytes are activated and infiltrate the alveolar tissue. In particular, alveolar macrophages play a key role in the progression of lung inflammation by producing cytokines and various MMPs, such as MMP-1, MMP-2, MMP-9, MMP-12, and MMP-14 (68,70). $CD4^+$ and $CD8^+$ T cells increase dramatically in the lungs of long-term smokers. Shifting toward a type 1 profile, these cells produces large amounts of IFN-γ, and release perforins and granzyme. In response to the massive accumulation of leukocytes, various inflammatory cytokines, such as IL-1α, IL-5, IL-6, and IL-18, as well as the chemokine monocyte chemotactic protein-1 and -3, macrophage inflammatory protein-1α, MIP-1β, MIP-1γ, MIP-2, MIP-3β, macrophage defined chemokine, granulocyte chemotactic protein-2, and interferon-γ–inducible protein-10 are increased in lung tissue of smoke-exposed mice (69,71). As a response, the accumulation of FoxP3 T regulatory cells in lungs is suggested to be a countermeasure to control the inflammation. Beside this direct disturbance of the pulmonary immune equilibrium, cell damage and the chronically activated immune system lead to a compromised immune status that is amplified by an increased mucosal permeability (69). Consequently, opportunistic pathogens cause infections in smokers, forcing inflammatory processes. In the long run, cellular damage leads to small airway remodeling in long-term smokers, which is characterized by mucus cell hyperplasia and peribronchiolar fibrosis, as well as increased airway smooth muscle mass (72).

Regarding the systemic inflammation of long-term cigarette smokers, it is hypothesized that these primarily local inflammatory processes in the pulmonary system tend to spill-over into the systemic circulation that serves as a mode of transit for inflammatory signals throughout the body. Thus, inflammation is suggested to be the main driver of pathophysiologic and degenerative processes in other tissues (Figure 2). In addition, smoke pollutants have been shown to directly cross through the alveolus-capillary interface. The particulate phase of cigarette smoke contains various lipophilic components, which are able to pass the lipid bilayer of the respiratory membranes, and therefore spread through the systemic bloodstream. Hence, these particles directly target specific organs where they might be recognized by receptors of the innate

immune system that initiate inflammatory signaling cascades through NF-κB activation (63,73).

3.2. Systemic inflammation during cigarette smoking

Acute cigarette smoking is followed by a temporary increase of systemic markers of oxidative stress, inflammation, and thrombosis, indicated by increased thiobarbituric acid reactive substances (TBARS), neutrophil elastase, leukotrienes, and leukocytes. In long-term smokers, these acute inflammatory processes induce tissue damage and turn into chronic inflammatory processes. (74,75). Lymphocytes turn into an activated phenotype indicated by an elevated expression of adhesion molecules, such as ICAM-1, VCAM-1, and E-selectin, which mediate the migration into the bronchoalveolar system or other tissues (76). These molecules can also be found in elevated quantities in their soluble form in heavy smokers (71). Regarding cytokines, the smoke-induced systemic low-grade inflammation is characterized by chronically elevated levels of markers for inflammation, tissue deterioration, and coagulation, such as C-reactive protein (CRP), TNF-α, von Willebrand factor, tissue inhibitor of metalloproteinases 1 (TIMP-1), factor VII, and fibrinogen (77).

The chronical enhancement of inflammation and oxidative stress in the blood, as well as some soluble components of cigarette smoke, disturb the function of endothelial cells. On the one hand, chronic inflammatory signals reduce nitric oxide (NO) production and vascular compliance, and are followed by aberrant interactions between endothelial and immune cells resulting from an increased expression of adhesion molecules. On the other hand, free radical formation from components of cigarette smoke and the activation of endogenous sources of free radicals such as uncoupled NOS, xanthine oxidase, and NADPH oxidase, further decrease NO availability, increasing coagulative factors and lipid peroxidation (75). Oxidative stress and inflammation are primary inducers of endothelial dysfunction, which represents an early hallmark in the development of atherosclerosis. Hence, after long-term smoking, dysfunctional endothelial cells express lower levels of prostacyclin, thrombomodulin, and tissue plasminogen activator (tPA), while expression levels of endothelin-1, angiotensin II, plasminogen activator inhibitor-1 (PAI-1), and von Willebrand factor (vWF) are increased (70,78).

In vitro studies proved that cigarette smoke also induces apoptosis, autophagic cell death, and necrosis in endothelial cells. In this regard, cigarette smoking decreases p53 and Bcl-2 expression, disrupts the vascular endothelial growth factor-(VEGF) and fluid shear stress-mediated VEGFR2/phosphoinositide 3-kinase (PI3K) signaling pathway, and reduces the cytochrome-c oxidase II expression through aberrant DNA methylation. Further vascular damage induced by excessive apoptosis was also shown to be initiated by a p53-independent caspase-3 activating pathways and protein carbonylation, which is caused by reactive oxygen species in cigarette smoking (79–81).

3.3. Effects of smoking on other organs and tissues

Toxic effects of cigarette smoke on the myocardium have been proven experimentally as well as clinically. It was demonstrated that smoking promotes neutrophil infiltration in the myocardium, alters T cell function, and causes DNA adducts in the myocardium (82) (Figure 2). Due to increased oxidative stress, ROS-sensitive signal transduction pathways (such as MAPKs) and various transcription factors (including NF-κB), are activated. This results in an aberrant cytokine profile (83,84). Gene analysis of the hearts of mice revealed an upregulation of the xenobiotic-metabolizing enzyme cytochrome P-450 1A1, and a downregulation of PAI-1, representing a key gene involved in fibrinolysis. These inflammatory processes are suggested to increase the risk for several diseases of the cardiovascular system in human smokers as well (85,86).

The muscles of smoke-exposed mice exhibited an increased expression of inflammatory cytokines, such as TNF-α and IL-1β (73). Furthermore, chronical exposure to cigarette smoke reduces muscle capillary-to-fiber ratio (76). Oxidative stress and inflammatory signals have been shown to activate the ubiquitin proteasome system, inhibit mitochondrial biogenesis, and reduce activation of the anabolic signaling pathways, such as the protein kinase B (Akt) and rapamycin (mTOR) pathways. Consequently, protein balance turns towards an enhanced degradation leading to muscle wasting, a reduced percentage of type I fiber, a lower muscle fiber cross-sectional area, and decreased muscle oxidative activity (73,87).

With regard to oxidative stress and inflammation, atherosclerosis and vascular brain lesions share similar pathological features (Figure 2). Accordingly, a higher expression of VEGF, ICAM-1, IL-8, and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) was also observed in cultured brain endothelial cells in response to smoke exposure (88). Cigarette smoke extracts induced heme oxygenase-1 (HO-1) expression mediated by the phosphatidolcholine phosopholipase C (PC-PLC)/protein kinase Cδ (PKCδ)/NADPH oxidase-dependent pathway and the platelet-derived growth factor receptor (PDGFR)/PI3K/Akt pathway (70). In rat brains exposed to cigarette smoke, endothelin-1 levels decreased, which is suggested an effect on hemodynamic responses (89). Since oxidative stress is known to play an important role in the pathogenesis of ischemic brain injury, cigarette smoking is associated with various cerebrovascular-related diseases, in particular, smoking is a risk factor for stroke (90).

Moreover, cigarette smoke negatively affected endothelial tight junctions, which was proved in animal experiments, and negatively affects the viability of the blood-brain-barrier. Smoke-induced neuroinflammation was confirmed by various in vivo studies using mouse and rat models. Mice exposed to cigarette smoke showed higher levels of ROS, induction of lipid peroxidation, activation of the transcription factors NF-κB and AP-1, as well as activation of MAPK, including JNK, ERK, p38, and COX -2 in various regions of the brain (90). In parallel, cigarette smoke altered enzymatic antioxidant defenses by reducing superoxide dismutase (SOD) as well as catalase and increasing glutathione S-transferase (GST) activity in rat brains. These alterations favor the proteolytic degradation of αII-spectrin through caspase-3 and the dephosphorylation of phosphoproteins enriched in astrocytes-15 (PEA-15), both indicating apoptotic cell death (91).

4. DIFFERENCES IN SMOKE-INDUCED INFLAMMATION AND INFLAMMATION DURING OBESITY

Both cigarette smoke and obesity-induced inflammation initiate a local inflammatory response, culminating in the recruitment of immune cells to the tissue in a feedback response trying to decrease this initial inflammation and avoid an exacerbation (77,92). While the source of inflammation in obese subjects seems to mainly be the visceral adipose tissue, inflammation after cigarette smoking originates in the pulmonary system. Accordingly, both inflammatory conditions represent risk factors for partly overlapping sets of diseases such as cardiovascular diseases, rheumatoid arthritis, hypertension, muscle wasting, and depressive disorders. However, there is a difference in other specific comorbidities (Figure 3). Regarding lung cancer, the proportion of cases attributable to smoking is estimated to about 90 percent in countries with a history of tobacco smoking. Similarly, smoking accounts for at least eight of ten COPD-related deaths (93), and the prevalence of tobacco use in idiopathic pulmonary fibrosis (IPF) is estimated in ranges from 41-83% (94). However, there are several other smoke-related diseases, such as coronary heart disease (CHD) or stroke, which at least partly progress due to the distribution of small particles and noxious gases throughout the body, directly triggering inflammatory processes. In contrast, obesity represents a specific risk factor for dyslipidemia, diabetes mellitus type II, various liver diseases (such as NAFLD), and certain cancers (such as colorectal and prostate cancer in men and endometrial, breast, and gallbladder cancer in women). However, both inflammatory conditions (i.e., obesity and long-term cigarette smoking) are characterized by a vicious cycle of local signals of cellular stress, inflammation, progressive immune cell infiltration, and the release of inflammatory cytokines and chemokines, leading to a spill-over into the circulation system. Subsequently, inflammatory processes target other tissues, which might account for various comorbidities. Obese smokers who combine both inflammatory disorders have been shown to have increased morbidity and mortality (73,92).

In the long-run, both obesity-induced inflammation and cigarette smoke-induced inflammation, are suggested to impair immune competence against invading pathogens. This condition of chronic immunosuppression seems to be much more pronounced in smokers due to significant impairment of the pulmonary barrier function (95). In obese patients, Neidich and collaborators (96) reported an increased risk of influenza among vaccinated adults who are obese, suggesting dysfunctional immunity. For both inflammatory conditions, it is suggested that chronic immune activation is a driver of immunosenescence. Accordingly, systemic inflammation was demonstrated to be a key element of the immune risk profile during aging, because chronic activation promotes the accumulation of senescent T cells that show a reduced reactivity against new invading pathogens (97).

While the pathogenesis of both inflammatory disorders have several features in common, there are also distinct differences in their development, extent, and the progression of immune activation (Figure 3). During cigarette smoking, the chronic exposure to toxic substances and inhaled particles seem to be important effectors of oxidative stress (75). Resulting severe cellular damage affects the function and integrity of airway epithelial cells (63). Subsequently, an important concomitant effect of cigarette smoke-induced inflammation is the significant increase of proteolytic peptidases, such as MMPs, which

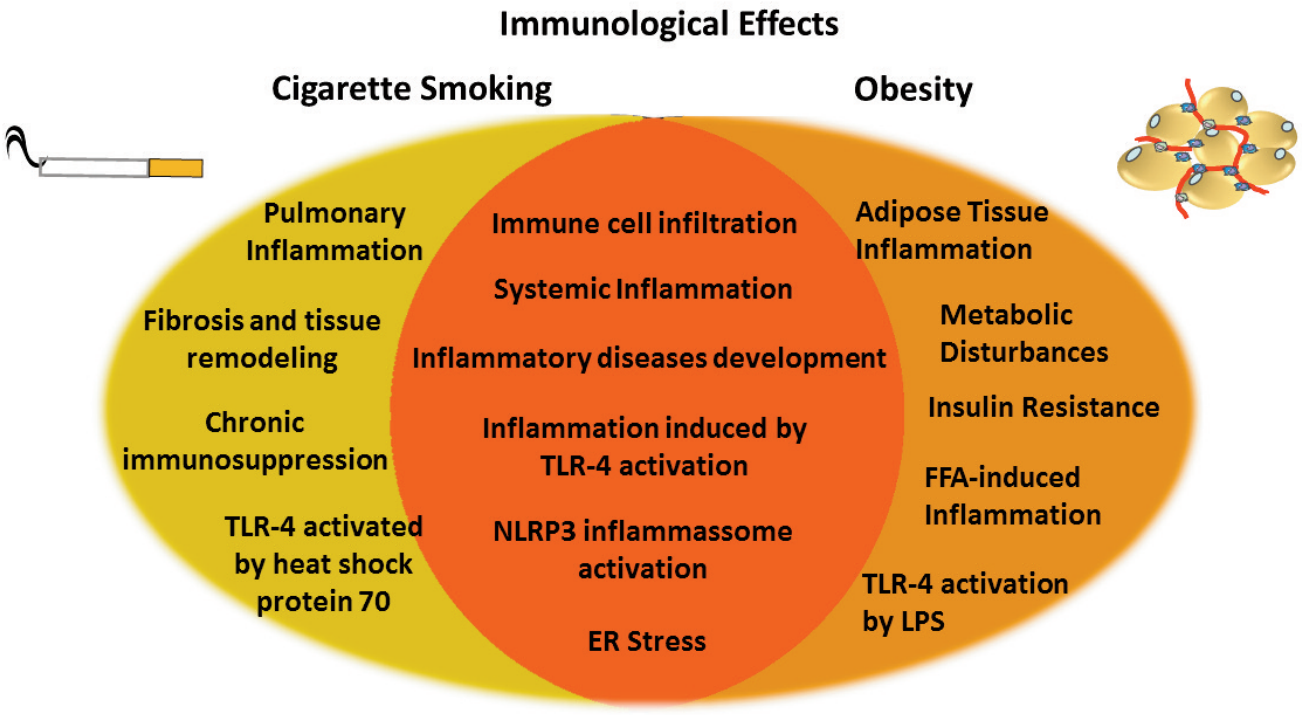


Figure 3: Specific inflammatory characteristics of cigarette smoke-induced inflammation (left), obesity-associated inflammation (right), and inflammatory characteristics both have in common (middle).

indicate progressive tissue deterioration and degradation. The resulting repair and remodeling processes induce fibrosis and the formation of excess fibrous connective tissue (68,69). Obesity is also accompanied by fibrotic process that occur in subcutaneous adipose tissue. The increase in collagen and extracellular matrix components induce an early insulin resistance and worsen the inflammation and cellular stress by reducing the adipocytes hypertrophy capacity (98). Adipose tissue fibrosis is strongly correlated with other obesity-associated comorbidities. Higher levels of fibrosis are found in a genetic model of lipodystrophy. These mice showed the same imbalance regarding insulin sensitivity, inflammation, and macrophage infiltration as found in obesity (99).

Another important feature of cigarette smoking is an enhanced platelet reactivity and increased blood coagulation, indicating an increased risk for thrombosis, peripheral artery occlusive disease (PAOD), and cardiovascular diseases (100). Since inflammatory processes in COPD patients often progress even after the cessation of smoking, and a combined accumulation of Th17 cells is observed in such cases, there are ongoing discussions concerning autoimmunity processes as potential drivers of disease progression in these cases (101).

An additional salient dissimilarity between cigarette smoking and obesity is that obesity is primarily characterized by metabolic disturbances. The greater accumulation of fatty acids within adipocytes changes the cell metabolic profile, increasing lipolysis and free fatty acids, which activate stress signals, inflammatory processes, and the production of inflammatory mediators by metabolic cells with secondary chemoattraction of immune cell infiltration (102).

An important pathway involved in this process is TLR4 signaling, since it is strongly activated by LPS and is required for free fatty acid-induced inflammation, which are both increased in obese subjects. The NRLP3 inflammasome also plays an important role in obesity-induced inflammation. Both TLR4, as well as NRLP3, can impair mitochondrial function in white adipose tissue adipocytes (103) and increase pro-inflammatory cytokine release. TLR4 activation in immune cells drives the cells to a pro-inflammatory phenotype exacerbating the tissue inflammation. Moreover, in the cross-talk between adipocytes and infiltrating immune cells, TLR4 upregulates MMP-1 expression in adipose tissue by increasing its expression in immune cells. This may contribute to the pathogenesis of obesity-induced inflammation (104). Similarly, cigarette smoke condensate has been shown to induce a macrophage pro-inflammatory response in vitro, which was dependent on MyD88, IL-1R1, and TLR4 signaling (105). In contrast to obesity, this pathway is not attributable to LPS. Instead, heat shock protein 70, which is also a TLR4 agonist, induces immune processes in the airways upon smoke exposure, which probably activates the innate immune system through TLR4/MyD88, resulting in airway inflammation (105).

Regarding molecular signaling, both smoke- and obesity-induced inflammation involve increased ER stress, followed by the activation of the innate immune response, which mainly affects adipocytes during obesity and airway epithelial cells in smokers (65,106). Similarly, both conditions are characterized by the activation of the NLRP3 inflammasome that is suggested to be an important driver of the pathogenesis of the specific diseases (66).

5. ANTI-INFLAMMATORY EFFECTS OF EXERCISE

It is well established that an active lifestyle can mitigate or counter the development of many metabolic and inflammatory diseases. For both obese patients and patients with pulmonary diseases, exercise training is an effective non-pharmacological treatment strategy. Exercise therapy increases strength, endurance capacity, and various quality of life scores. Recent studies provided evidence that regular and moderate exercise exerts protective effects due to its immune-regulating properties. In this regard, observational and interventional studies have shown that regular exercise training is able to reduce circulating inflammatory markers in healthy and diseased individuals (107). Due to the differences in the inflammatory pathogenesis of both diseases, the immune-regulating effects of exercise may differ between them. These differences might at least have implications for the type, impact, and success of exercise as a treatment strategy for these chronic inflammatory diseases.

5.1. Immune-regulatory effects of exercise during obesity

Since obesity is a metabolic disease caused by exacerbated fat accumulation, alterations in metabolic pathways, and activation of inflammatory pathways, strategies to increase fat oxidation, restore metabolism homeostasis, and decrease inflammation have achieved success in obesity treatment.

Several studies, in humans and rodents, provide evidence that regular exercise can decrease fat mass accumulation (77), even after high fat-diet feeding. The exercise-induced loss of body fat is associated with the improvement of many obesity-related disorders, such as insulin resistance, cardiovascular disease, and NASH (46).

The metabolic improvement in obese subjects resulting from exercise could be explained on the one hand by increases in fatty acid mobilization in adipose tissue, leading to a reduction in cellular stress. These fatty acids are, therefore, oxidized by skeletal muscle to produce energy, which decreases the “toxic” free fatty acids in circulation.

Exercise training has also been shown to reduce adipose tissue inflammation. Three months of an aerobic exercise program decreased IL-6 and TNF-α protein expression in the subcutaneous adipose tissue of diabetic and non-diabetic obese patients (108). The main mechanisms behind this anti-inflammatory role of exercise include a reduction in immune cell infiltration and a shift in the pro-inflammatory profile (found in obese adipose tissue) to an anti-inflammatory one (found in lean adipose tissue). Human studies have found that exercise training can decrease M1 markers in circulating monocytes and increase M2 expression. Eight weeks of low-intensity exercise training was sufficient to increase CD14 and AMAC-1 (M2 markers) gene expression and decrease CXCL2 in the

peripheral blood mononuclear cells of sedentary individuals (109). A short-term period (two weeks) of moderate-intensity continuous training reduced CCR2 and CXCR2 mRNA in circulating monocytes from obese adults, while high-intensity interval training increased CCR5 expression (110), suggesting that moderate exercise provides anti-inflammatory effects on monocytes.

Furthermore, the aerobic training is able to modify adaptive immune responses (111). The subsets of lymphocytes are altered by acute or chronic exercise, and these alterations depend on the intensity and duration of exercise, pre-exercise status of training and energetic substrate availability. Athletes who exercise at higher intensity and for long durations (e.g., marathoners, triathletes and cyclists) showed increased in Treg lymphocytes in the systemic circulation (112). Moreover, CD4+ T cells positive to TGF-β and IL-10 were increased in marathon runners (113).

In studies with animals, Kawanishi and collaborators (114) found that twelve weeks of treadmill exercise in obese mice reduced adipose tissue TNF-α and IL-6 gene expression, followed by a reduction in the percentage and the number of macrophages and CD8 T cells in the stromal vascular fraction (SVF). These macrophages also presented a decreased CD11c surface expression, which represents a marker of M1 macrophages (114). Similarly, four months of exercise training reduced adipose tissue inflammation, macrophage infiltration, and increased gene expression of CD163 – a marker for M2 macrophages (115). This suggests that exercise can decrease a macrophage inflammatory phenotype, as well as decrease macrophage infiltration, which restored the obesity-induced adipose tissue inflammation. In a recent paper, our group demonstrated that 8 weeks of treadmill training (60 min at 60% of maximal velocity, 5 days a week) was able to induce the M2 phenotype in macrophages from subcutaneous adipose tissue, even in those mice lacking PPARγ in the myeloid cell lineage (116). PPARγ is one of the main regulatory nuclear transcription factors for M2 cells. In this study, we also observed an increase in IL-10 production by stimulated peritoneal macrophages from trained mice (116). These findings indicate that exercise can induce an anti-inflammatory response in macrophages by mechanisms independent of PPARγ.

Studies on animals fed a high-fat diet showed that endurance exercise training decreased expression of TLR-4 in monocytes, adipose tissue, and bone-marrow-derived macrophages, followed by a reduced TLR-4/MyD88 interaction (117). Humans studies revealed that obese people submitted to an exercise program showed low levels of TLR-4 in peripheral-blood mononuclear cells (117). A short-term program (two weeks) of both intense and moderate exercise decreased TLR4 expression in the circulating leucocytes of sedentary adults (118).

Targeting TLR-4 is an important step to control NF-κB activation and pro-inflammatory cytokine transcription, which represents an important pathway for obesity-induced insulin resistance. Several studies have reported that different types of exercise can inhibit the activation of the NF-κB pathway, followed by decreased inflammation and restored insulin signaling (119). Moreover, exercise training decreases endotoxaemia by improving gut barrier function (120). LPS is the most potent TLR-4 ligand, decreasing the LPS concentration in the serum implies an inhibition of TLR-4 signaling, thus decreasing NF-κB activation.

These alterations in immune cells induced by exercise can be, in part, modulated by muscle mass contraction. Pedersen and collaborators (121) showed that during exercise muscle mass increases the capacity to produce cytokines, indicating that muscle is an important immune-modulator organ. IL-6 is elevated in serum after an acute bout of aerobic exercise. Muscle-derived IL-6, together with IL-10 and IL-1ra expression by leukocytes, represent important anti-inflammatory cytokines that inhibit IL-1 and TNF-a signaling and improve insulin sensitivity. Moreover, IL-6 has been shown to enhance lipolysis and fatty acid oxidation in the muscle and adipose tissue, as well as increase glucose uptake in an AMPK-dependent manner (3).

IL-15, which is upregulated in the skeletal muscle of trained people (122), may also play a role in reducing fat mass. It reduced visceral fat in mice (123). Plasma levels of IL-15 are inversely correlated with visceral fat amount in obese people (123). Furthermore, IL-15 induces different NK and T cell subtype differentiation, and inhibits cell apoptosis (124,125).

5.2. Anti-inflammatory effects of exercise after smoke exposure

Despite the ever increasing number of patients suffering from smoke-induced diseases such as COPD, current treatments can only decelerate, not prevent, the progression of the diseases (126). Often, these patients are limited in their ability to perform exercises. However, after some weeks of exercise training, they benefit by improving their strength, cardiopulmonary fitness, and quality of life. In response to regular physical activity, some human and murine studies also proved immune-regulating effects. In humans, a reduction of systemic CRP and IL-6 levels in response to physical activity has been shown (127). In smoke-exposed mice, regular treadmill running was followed by lower levels of inflammatory, chemoattractive, and coagulative proteins in the blood (73). Regarding blood coagulation, a reduction of von Willebrand factor and Factor VII has been shown. Similarly, a reduced surface expression of adhesion molecules on circulating lymphocytes such as VCAM-1, ICAM-1 and CD62L, has been shown after regular treadmill running. In parallel, several inflammatory cytokines such as IL-1α, MCP-3, MIP1β, MIP-1α, and CD40L decreased after exercise in plasma. Exercise also decreased the rate of leucine appearance, suggesting an attenuation of accelerated whole-body protein breakdown in patients with COPD (128).

Physical training does not improve lung function in patients with COPD. Accordingly, increased cardiorespiratory fitness is mainly attributed to improvements of muscle and cardiac function. Animal studies suggest that regular exercise training is able to at least partly reduce lung inflammation and decelerate the remodeling of lung tissue. Indeed, exercise increased Th1 responses and suppressed Th2 cytokine levels in the

lungs of mice after smoke exposure (129). These immune-regulating effects might be due to an increased antioxidant defense after training, which was demonstrated by a reduction of oxidative stress markers (130). Prior exercise training has been shown to alleviate markers of lung inflammation and lung remodeling induced by subsequent exposure to environmental cigarette smoke. Accordingly, a significant reduction of inflammatory cell infiltration, cytokines, chemokines, adhesion molecules, activation of NF-κB was demonstrated, accompanied by a reduced bronchoalveolar-capillary permeability and epithelial thickening (131).

There are only limited data available about the effects of exercise on the endothelium in smokers. In mice it was shown that exercise is able to reverse peripheral endothelial dysfunction after smoke exposure by increasing maximal endothelium-dependent dilation. In parallel, protein expression of phosphorylated eNOS was increased in the aorta of trained mice (132). Endothelial dysfunction and cardiac dysfunction are strongly connected. Accordingly, it is assumed that the protective effects of exercise towards the endothelium might also be cardioprotective (133,134). Bowen et al. (132) proved that right ventricular dysfunction after smoke exposure can be reversed by exercise training, while the underlying mechanisms are not known. Exercise training has been shown to reverse various aspects of muscle wasting after long-term smoking. In the muscle of smoke-exposed mice, a decrease in inflammatory signals such as TNF-α and IL-1β on RNA level has been reported after training (73). The anti-inflammatory effects are suggested to involve blunting of the activation of catabolic pathways, such as the ubiquitin proteasome system. In this context, exercise decreased FoxO1 phosphorylation, reduced the expression of atrogin-1 and MuRF-1, and abrogated the expression of protein catabolic E3 ligases in muscle, which are considered key factors in myofibrillar protein breakdown via the ubiquitin proteasome system. Similarly, exercise increases anabolic signaling after smoke exposure by increasing IGF-1 signaling and activation of the Akt–mTOR–pathway in muscle tissue. Exercise stimulates the metabolic capacities of muscles after smoke exposure. Thus, an increased expression of genes involved in fatty acid transport into the mitochondrial matrix and an increase of glucose uptake was observed in smoke exposed of mice after training (73).

6. DIFFERENTIAL IMMUNOLOGIC REGULATION OF EXERCISE DURING OBESITY AND AFTER SMOKE EXPOSURE

The impact of exercise training as a therapeutic strategy for obese patients excluded this patients or for patients with pulmonary diseases might at first be strongly dependent on the progression or pathologic stage of the disease. However, in the wide array of smoke-induced pulmonary diseases the effects of therapeutic training are limited. On the one hand, COPD patients at least patients in stage III or IV—are only able to take part in effective types of exercise training programs in a limited fashion (135). On the other hand, exercise has neither the potential to regenerate lung tissue nor to improve lung function significantly (129). Instead, regular exercise training mainly alters the function of peripheral tissues such as cardiac function or muscles. Some studies have

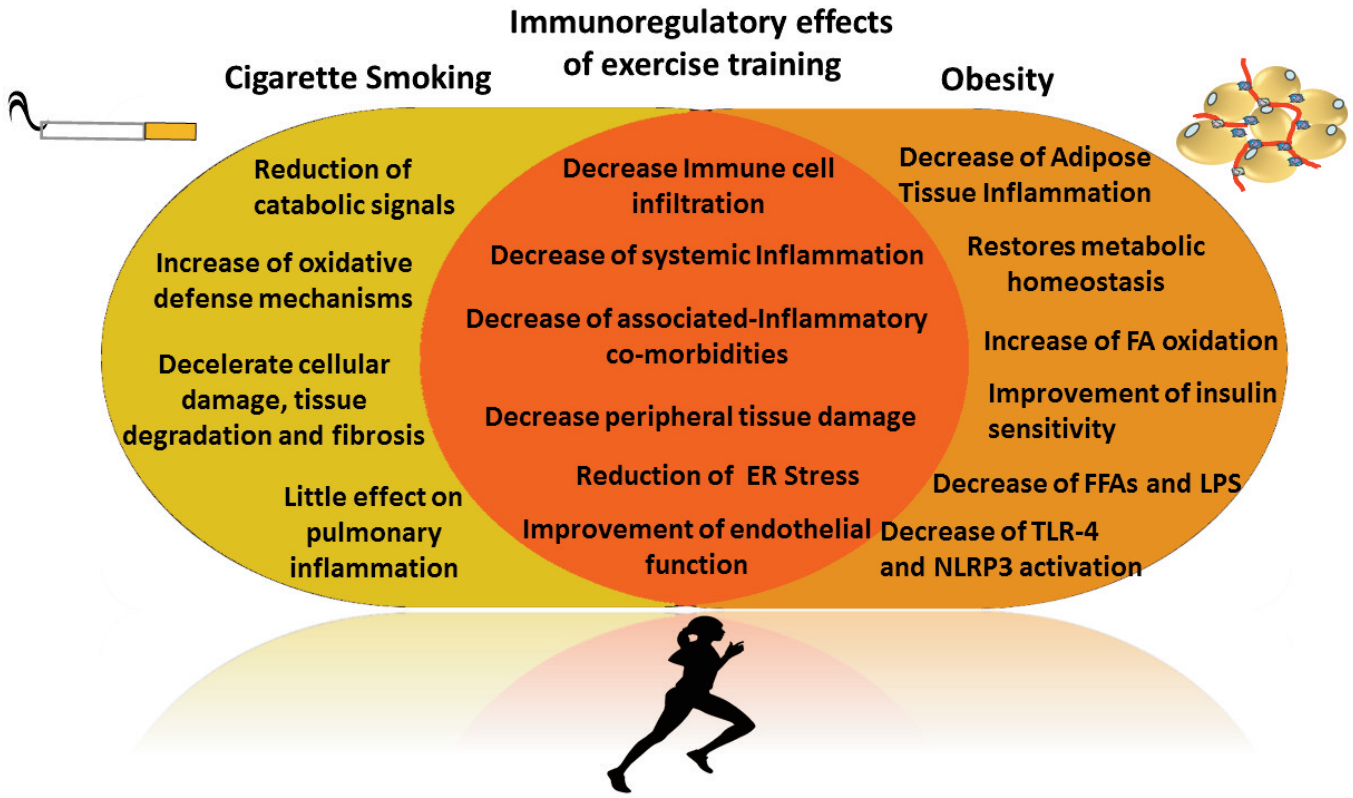


Figure 4: Specific effects of regular exercise training on cigarette smoke-induced inflammation (left side) and obesity associated inflammation (right side) and common effects (middle).

demonstrated muscular dysfunction, indicated by anabolic resistance, in response to training (136). However, this process might depend on the progress of the disease, because there is also some evidence for a reduction of inflammation and oxidative stress followed by increased activation of anabolic signals, decrease of catabolic pathways, strength, and endurance capacity (73). Regarding tissue degradation, it is suggested that the immune-regulating effects of exercise mainly decelerate or stabilize the level progression of cellular damage and fibrosis (73,92) (Figure 4).

During obesity, however, exercise appears to have a much more pronounced effect. Since obesity is characterized by strong metabolic alterations in adipose tissue and other organs, many pathways are involved in obesity-induced inflammation, thus exercise can act in different ways to restore body homeostasis and reduce inflammatory responses. Exercise training induces fatty acid oxidation, which reduces free fatty acids. Moreover, trained human and rodents presented lower LPS concentration in the serum (137,138). Combining these factors culminates in a decrease of TLR4 and NLRP3 activation, which reduces immune cell infiltration into the adipose tissue, inhibits the pro-inflammatory phenotype and decreases local and systemic inflammation (139). Furthermore, exercise can restore glucose homeostasis and insulin sensitivity in many different organs and tissues. Recent work reported that adipose-specific insulin resistance increases MCP-1 expression and M1 macrophage infiltration into the adipose tissue (140). Therefore, restoring insulin signaling is an important pathway to decreasing obesity-induced inflammation (Figure 4).

Several studies have reported that the effects of exercise on obesity are independent of an alterations in fat mass index or high-fat diet consumption (114,115,141). In many studies, there is no nutritional intervention; they only add exercise to the routine, and the effects include reduced inflammation, restored glucose and insulin signaling, and decreased obesity-associated comorbidities (46,141). So, many years of research in the obesity field lead us to believe that the problem facing Western society is not the high consumption of fat by itself, but the combination of this with a sedentary lifestyle (142,143). In contrast, smoke-induced inflammation develops independently of a sedentary lifestyle. There is no consistent association between physical inactivity and pulmonary function in adult smokers (144). Accordingly, regular smoking is suggested to represent an inflammatory trigger that cannot be completely compensated for by regular exercise. Consequently, smoking history and smoking cessation should be given special attention in prevention and therapeutic strategies.

7. CONCLUSION

Inflammation during obesity or after cigarette smoking has several aspects in common, but there are also distinct differences in their pathogenesis. Accordingly, it is reasonable to talk about an obesity-type of chronic low-grade inflammation and a cigarette smoke-type of systemic inflammation. Disease-specific severity, localization, and tissue-specific immunologic signaling pathways reflect some of the main differences between both inflammatory diseases. Downstream, smoking and obesity share some hallmarks of inflammation that affect the risk for various inflammatory comorbidities, such as cardiovascular disease and rheumatoid arthritis (2). It is worth mentioning that there is a proportion of patients who combine obesity and smoking, and the interaction between both resulting inflammatory processes increases the overall risk for morbidity and mortality. Regarding the immune-regulating effects of exercise, it is important the note that exercise training represents a holistic therapeutic approach that affects both local as well as systemic inflammatory processes in obese patients and long-term cigarette smokers. However, in cases of advanced pulmonary damage after long-term cigarette smoking, the immunological effects of therapeutic exercise are limited, which makes it important to start therapeutic training as soon as possible.

Accordingly, exercise training represents an integrated therapeutic approach with only limited negative side effects (145). But due to the distinctive differences between obesity and cigarette smoking, future studies should consider investigating the immune-regulating exercise strategies that more specifically target disease-specific inflammation.

8. REFERENCES

1. Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, et al. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes. 2003 Jul;52(7):1799–805.
2. de Hair MJH, Landewé RBM, van de Sande MGH, van Schaardenburg D, van Baarsen LGM, Gerlag DM, et al. Smoking and overweight determine the likelihood of developing rheumatoid arthritis. Ann Rheum Dis. 2013 Oct;72(10):1654–8.
3. Pedersen BK. Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. Eur J Clin Invest. 2017 Aug;47(8):600–11.
4. Gea J, Casadevall C, Pascual S, Orozco-Levi M, Barreiro E. Clinical management of chronic obstructive pulmonary disease patients with muscle dysfunction. J Thorac Dis. 2016 Nov;8(11):3379–400.
5. GHO | By category | Prevalence of overweight among adults, BMI ≥ 25, age-standardized - Estimates by WHO Region [Internet]. [cited 2018 Sep 27]. Available from: http://apps.who.int/gho/data/view.main.GLOBAL2461A?lang=en
6. Peeters A, Barendregt JJ, Willekens F, Mackenbach JP, Al Mamun A, Bonneux L, et al. Obesity in adulthood and its consequences for life expectancy: a life-table analysis. Ann Intern Med. 2003 Jan 7;138(1):24–32.
7. Jimenez MP, Green MA, Subramanian SV, Razak F. A demographic, clinical, and behavioral typology of obesity in the United States: an analysis of National Health and Nutrition Examination Survey 2011-2012. Ann Epidemiol. 2018 Mar;28(3):175-181.e4.
8. Spiegelman BM, Hotamisligil GS. Through thick and thin: wasting, obesity, and TNF alpha. Cell. 1993 May 21;73(4):625–7.
9. Hammond RA, Levine R. The economic impact of obesity in the United States. Diabetes Metab Syndr Obes. 2010 Aug 30;3:285–95.

10. Unamuno X, Gómez-Ambrosi J, Rodríguez A, Becerril S, Frühbeck G, Catalán V. Adipokine dysregulation and adipose tissue inflammation in human obesity. Eur J Clin Invest. 2018 Sep;48(9):e12997.
11. Lancaster GI, Febbraio MA. The immunomodulating role of exercise in metabolic disease. Trends Immunol. 2014 Jun;35(6):262–9.
12. Cox AJ, West NP, Cripps AW. Obesity, inflammation, and the gut microbiota. Lancet Diabetes Endocrinol. 2015 Mar;3(3):207–15.
13. Collins KH, Herzog W, MacDonald GZ, Reimer RA, Rios JL, Smith IC, et al. Obesity, metabolic syndrome, and musculoskeletal disease: Common inflammatory pathways suggest a central role for loss of muscle integrity. Front Physiol. 2018;9:112.
14. Fuentes E, Fuentes F, Vilahur G, Badimon L, Palomo I. Mechanisms of chronic state of inflammation as mediators that link obese adipose tissue and metabolic syndrome. Mediators Inflamm. 2013;2013:136584.
15. Cinti S. Adipose organ development and remodeling. Compr Physiol. 2018 Sep 14;8(4):1357–431.
16. Mandal P, Pratt BT, Barnes M, McMullen MR, Nagy LE. Molecular Mechanism for Adiponectin-dependent M2 Macrophage Polarization link between the metabolic and innate immune activity of full-length adiponectin. J Biol Chem. 2011 Apr 15;286(15):13460–9.
17. Lira FS, Rosa JC, Pimentel GD, Seelaender M, Damaso AR, Oyama LM, et al. Both adiponectin and interleukin-10 inhibit LPS-induced activation of the NF-κB pathway in 3T3-L1 adipocytes. Cytokine. 2012 Jan;57(1):98–106.
18. Wensveen FM, Valentić S, Šestan M, Turk Wensveen T, Polić B. The "Big Bang" in obese fat: Events initiating obesity-induced adipose tissue inflammation. Eur J Immunol. 2015 Sep;45(9):2446–56.
19. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest. 2007 Jan;117(1):175–84.
20. Miller AM, Asquith DL, Hueber AJ, Anderson LA, Holmes WM, McKenzie AN, et al. Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. Circ Res. 2010 Sep 3;107(5):650–8.
21. Teixeira AA de S, Lira FS, Pimentel GD, Oliveira de Souza C, Batatinha H, Biondo LA, et al. Aerobic exercise modulates the free fatty acids and inflammatory response during obesity and cancer cachexia. Crit Rev Eukaryot Gene Expr. 2016;26(3):187–98.
22. Klöting N, Blüher M. Adipocyte dysfunction, inflammation and metabolic syndrome. Rev Endocr Metab Disord. 2014 Dec;15(4):277–87.
23. Blüher M, Bashan N, Shai I, Harman-Boehm I, Tarnovscki T, Avinaoch E, et al. Activated Ask1-MKK4-p38MAPK/JNK stress signaling pathway in human omental fat tissue may link macrophage infiltration to whole-body Insulin sensitivity. J Clin Endocrinol Metab. 2009 Jul;94(7):2507–15.
24. Verboven K, Wouters K, Gaens K, Hansen D, Bijnen M, Wetzels S, et al. Abdominal subcutaneous and visceral adipocyte size, lipolysis and inflammation relate to insulin resistance in male obese humans. Sci Rep. 2018 Mar 16;8(1):4677.
25. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol. 2011;29:415–45.
26. Martinon F, Gaide O, Pétrilli V, Mayor A, Tschopp J. NALP inflammasomes: a central role in innate immunity. Semin Immunopathol. 2007 Sep;29(3):213–29.
27. Lee B-C, Lee J. Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. Biochim Biophys Acta. 2014 Mar;1842(3):446–62.
28. Mathis D. Immunological goings-on in visceral adipose tissue. Cell Metab. 2013 Jun 4;17(6):851–9.
29. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003 Dec;112(12):1796–808.
30. Amano SU, Cohen JL, Vangala P, Tencerova M, Nicoloro SM, Yawe JC, et al. Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation. Cell Metab. 2014 Jan 7;19(1):162–71.
31. Wouters K, Gaens K, Bijnen M, Verboven K, Jocken J, Wetzels S, et al. Circulating classical monocytes are associated with CD11c+ macrophages in human visceral adipose tissue. Sci Rep. 2017 15;7:42665.
32. Patsouris D, Li P-P, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metab. 2008 Oct;8(4):301–9.
33. Bijnen M, Josefs T, Cuijpers I, Maalsen CJ, van de Gaar J, Vroomen M, et al. Adipose tissue macrophages induce hepatic neutrophil recruitment and macrophage accumulation in mice. Gut. 2018;67(7):1317–27.
34. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev. 2009 Apr;22(2):240–73.
35. Orr JS, Puglisi MJ, Ellacott KLJ, Lumeng CN, Wasserman DH, Hasty AH. Toll-like receptor 4 deficiency promotes the alternative activation of adipose tissue macrophages. Diabetes. 2012 Nov;61(11):2718–27.
36. Griffin C, Eter L, Lanzetta N, Abrishami S, Varghese M, McKernan K, et al. TLR4, TRIF, and MyD88 are essential for myelopoiesis and CD11c+ adipose tissue macrophage production in obese mice. J Biol Chem. 2018 Jun 8;293(23):8775–86.
37. Singer K, DelProposto J, Morris DL, Zamarron B, Mergian T, Maley N, et al. Diet-induced obesity promotes myelopoiesis in hematopoietic stem cells. Mol Metab. 2014 Sep;3(6):664–75.
38. Razolli DS, Moraes JC, Morari J, Moura RF, Vinolo MA, Velloso LA. TLR4 expression in bone marrow-derived cells is both necessary and sufficient to produce the insulin resistance phenotype in diet-induced obesity. Endocrinology. 2015 Jan;156(1):103–13.
39. Pierre N, Deldicque L, Barbé C, Naslain D, Cani PD, Francaux M. Toll-like receptor 4 knockout mice are protected against endoplasmic reticulum stress induced by a high-fat diet. PLoS ONE. 2013;8(5):e65061.
40. Ghosh AK, O'Brien M, Mau T, Yung R. Toll-like receptor 4 (TLR4) deficient mice are protected from adipose tissue inflammation in aging. Aging (Albany NY). 2017 Sep 7;9(9):1971–82.
41. Lancaster GI, Langley KG, Berglund NA, Kammoun HL, Reibe S, Estevez E, et al. Evidence that TLR4 is not a receptor for saturated fatty acids but mediates lipid-induced inflammation by reprogramming macrophage metabolism. Cell Metab. 2018 May 1;27(5):1096-1110.e5.

42. Pendyala S, Walker JM, Holt PR. A high-fat diet is associated with endotoxemia that originates from the gut. Gastroenterology. 2012 May;142(5):1100-1101.e2.
43. Genser L, Aguanno D, Soula HA, Dong L, Trystram L, Assmann K, et al. Increased jejunal permeability in human obesity is revealed by a lipid challenge and is linked to inflammation and type 2 diabetes. J Pathol. 2018 Oct;246(2):217–30.
44. Satapathy SK, Sanyal AJ. Epidemiology and natural history of nonalcoholic fatty liver disease. Semin Liver Dis. 2015 Aug;35(3):221–35.
45. de Souza CO, Teixeira AAS, Biondo LA, Lima Junior EA, Batatinha HAP, Rosa Neto JC. Palmitoleic acid improves metabolic functions in fatty liver by PPARα-dependent AMPK activation. J Cell Physiol. 2017 Aug;232(8):2168–77.
46. Batatinha HAP, Lima EA, Teixeira AAS, Souza CO, Biondo LA, Silveira LS, et al. Association between aerobic exercise and rosiglitazone avoided the NAFLD and liver inflammation exacerbated in PPAR-α knockout mice. J Cell Physiol. 2017 May;232(5):1008–19.
47. Tiniakos DG, Vos MB, Brunt EM. Nonalcoholic fatty liver disease: pathology and pathogenesis. Annu Rev Pathol. 2010;5:145–71.
48. Ogawa Y, Imajo K, Honda Y, Kessoku T, Tomeno W, Kato S, et al. Palmitate-induced lipotoxicity is crucial for the pathogenesis of nonalcoholic fatty liver disease in cooperation with gut-derived endotoxin. Sci Rep. 2018 Jul 27;8(1):11365.
49. Ma C, Zhang Q, Greten TF. Nonalcoholic fatty liver disease promotes hepatocellular carcinoma through direct and indirect effects on hepatocytes. FEBS J. 2018 Feb;285(4):752–62.
50. Park BS, Song DH, Kim HM, Choi B-S, Lee H, Lee J-O. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. Nature. 2009 Apr 30;458(7242):1191–5.
51. Khan HA, Ahmad MZ, Khan JA, Arshad MI. Crosstalk of liver immune cells and cell death mechanisms in different murine models of liver injury and its clinical relevance. Hepatobiliary Pancreatic Dis Int. 2017 Jun;16(3):245–56.
52. de Souza CO, Valenzuela CA, Baker EJ, Miles EA, Rosa Neto JC, Calder PC. Palmitoleic acid has stronger anti-inflammatory potential in human endothelial cells compared to oleic and palmitic acids. Mol Nutr Food Res. 2018 Aug 13;e1800322.
53. Melo LG, Gnecchi M, Pachori AS, Kong D, Wang K, Liu X, et al. Endothelium-targeted gene and cell-based therapies for cardiovascular disease. Arterioscler Thromb Vasc Biol. 2004 Oct;24(10):1761–74.
54. Artwohl M, Roden M, Waldhäusl W, Freudenthaler A, Baumgartner-Parzer SM. Free fatty acids trigger apoptosis and inhibit cell cycle progression in human vascular endothelial cells. FASEB J. 2004 Jan;18(1):146–8.
55. Warfel JD, Bermudez EM, Mendoza TM, Ghosh S, Zhang J, Elks CM, et al. Mitochondrial fat oxidation is essential for lipid-induced inflammation in skeletal muscle in mice. Sci Rep. 2016 28;6:37941.
56. Morino K, Petersen KF, Sono S, Choi CS, Samuel VT, Lin A, et al. Regulation of mitochondrial biogenesis by lipoprotein lipase in muscle of insulin-resistant offspring of parents with type 2 diabetes. Diabetes. 2012 Apr;61(4):877–87.
57. Hussey SE, Lum H, Alvarez A, Cipriani Y, Garduño-Garcia J, Anaya L, et al. A sustained increase in plasma NEFA upregulates the Toll-like receptor network in human muscle. Diabetologia. 2014 Mar;57(3):582–91.
58. Cialdella-Kam L, Ghosh S, Meaney MP, Knab AM, Shanely RA, Nieman DC. Quercetin and green tea extract supplementation downregulates genes related to tissue inflammatory responses to a 12-week high fat-diet in mice. Nutrients. 2017 Jul 19;9(7).
59. Ng M, Freeman MK, Fleming TD, Robinson M, Dwyer-Lindgren L, Thomson B, et al. Smoking prevalence and cigarette consumption in 187 countries, 1980-2012. JAMA. 2014 Jan 8;311(2):183–92.
60. Edwards R. The problem of tobacco smoking. BMJ. 2004 Jan 24;328(7433):217–9.
61. van der Vaart H, Postma DS, Timens W, ten Hacken NHT. Acute effects of cigarette smoke on inflammation and oxidative stress: a review. Thorax. 2004 Aug;59(8):713–21.
62. Pauwels RA, Buist AS, Ma P, Jenkins CR, Hurd SS, GOLD Scientific Committee. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: National heart, lung, and blood institute and world health organization global initiative for chronic obstructive lung disease (GOLD): executive summary. Respir Care. 2001 Aug;46(8):798–825.
63. Zhang J, Liu Y, Shi J, Larson DF, Watson RR. Side-stream cigarette smoke induces dose-response in systemic inflammatory cytokine production and oxidative stress. Exp Biol Med (Maywood). 2002 Oct;227(9):823–9.
64. Pouwels SD, Hesse L, Faiz A, Lubbers J, Bodha PK, Ten Hacken NHT, et al. Susceptibility for cigarette smoke-induced DAMP release and DAMP-induced inflammation in COPD. Am J Physiol Lung Cell Mol Physiol. 2016 01;311(5):L881–92.
65. Wang Y, Wu Z-Z, Wang W. Inhibition of endoplasmic reticulum stress alleviates cigarette smoke-induced airway inflammation and emphysema. Oncotarget. 2017 Sep 29;8(44):77685–95.
66. Birrell MA, Eltom S. The role of the NLRP3 inflammasome in the pathogenesis of airway disease. Pharmacol Ther. 2011 Jun;130(3):364–70.
67. Crotty Alexander LE, Shin S, Hwang JH. Inflammatory Diseases of the Lung Induced by Conventional Cigarette Smoke: A Review. Chest. 2015 Nov;148(5):1307–22.
68. Tang G-J, Wang H-Y, Wang J-Y, Lee C-C, Tseng H-W, Wu Y-L, et al. Novel role of AMP-activated protein kinase signaling in cigarette smoke induction of IL-8 in human lung epithelial cells and lung inflammation in mice. Free Radic Biol Med. 2011 Jun 1;50(11):1492–502.
69. Botelho FM, Gaschler GJ, Kianpour S, Zavitz CCJ, Trimble NJ, Nikota JK, et al. Innate immune processes are sufficient for driving cigarette smoke-induced inflammation in mice. Am J Respir Cell Mol Biol. 2010 Apr;42(4):394–403.
70. Shih R-H, Cheng S-E, Hsiao L-D, Kou YR, Yang C-M. Cigarette smoke extract upregulates heme oxygenase-1 via PKC/NADPH oxidase/ROS/PDGFR/PI3K/Akt pathway in mouse brain endothelial cells. J Neuroinflammation. 2011 Aug 24;8:104.
71. Rusznak C, Mills PR, Devalia JL, Sapsford RJ, Davies RJ, Lozewicz S. Effect of cigarette smoke on the permeability and IL-1beta and sICAM-1 release from cultured human bronchial epithelial cells of never-smokers, smokers, and patients with chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol. 2000 Oct;23(4):530–6.

72. Hogg JC, Timens W. The pathology of chronic obstructive pulmonary disease. Annu Rev Pathol. 2009;4:435–59.
73. Krüger K, Seimetz M, Ringseis R, Wilhelm J, Pichl A, Couturier A, et al. Exercise training reverses inflammation and muscle wasting after tobacco smoke exposure. Am J Physiol Regul Integr Comp Physiol. 2018 Mar 1;314(3):R366–76.
74. Wannamethee SG, Lowe GDO, Shaper AG, Rumley A, Lennon L, Whincup PH. Associations between cigarette smoking, pipe/cigar smoking, and smoking cessation, and haemostatic and inflammatory markers for cardiovascular disease. Eur Heart J. 2005 Sep;26(17):1765–73.
75. Liu J, Liang Q, Frost-Pineda K, Muhammad-Kah R, Rimmer L, Roethig H, et al. Relationship between biomarkers of cigarette smoke exposure and biomarkers of inflammation, oxidative stress, and platelet activation in adult cigarette smokers. Cancer Epidemiol Biomarkers Prev. 2011 Aug;20(8):1760–9.
76. Krüger K, Dischereit G, Seimetz M, Wilhelm J, Weissmann N, Mooren FC. Time course of cigarette smoke-induced changes of systemic inflammation and muscle structure. Am J Physiol Lung Cell Mol Physiol. 2015 Jul 15;309(2):L119-128.
77. Krüger K, Mooren FC, Eder K, Ringseis R. Immune and inflammatory signaling pathways in exercise and obesity. Am J Lifestyle Med. 2016 Aug;10(4):268–79.
78. Ribeiro F, Alves AJ, Duarte JA, Oliveira J. Is exercise training an effective therapy targeting endothelial dysfunction and vascular wall inflammation? Int J Cardiol. 2010 Jun 11;141(3):214–21.
79. Yang Y-M, Liu G-T. Damaging effect of cigarette smoke extract on primary cultured human umbilical vein endothelial cells and its mechanism. Biomed Environ Sci. 2004 Jun;17(2):121–34.
80. Edirisinghe I, Rahman I. Cigarette smoke-mediated oxidative stress, shear stress, and endothelial dysfunction: role of VEGFR2. Ann N Y Acad Sci. 2010 Aug;1203:66–72.
81. Wang J, Wilcken DE, Wang XL. Cigarette smoke activates caspase-3 to induce apoptosis of human umbilical venous endothelial cells. Mol Genet Metab. 2001 Jan;72(1):82–8.
82. Das A, Dey N, Ghosh A, Das S, Chattopadhyay DJ, Chatterjee IB. Molecular and cellular mechanisms of cigarette smoke-induced myocardial injury: prevention by vitamin C. PLoS ONE. 2012;7(9):e44151.
83. Zhou X, Li C, Xu W, Chen J. Protective effects of valsartan against cigarette smoke-induced left ventricular systolic dysfunction in rats. Int J Cardiol. 2013 Aug 10;167(3):677–80.
84. Khanna AK, Xu J, Mehra MR. Antioxidant N-acetyl cysteine reverses cigarette smoke-induced myocardial infarction by inhibiting inflammation and oxidative stress in a rat model. Lab Invest. 2012 Feb;92(2):224–35.
85. McCall MR, van den Berg JJ, Kuypers FA, Tribble DL, Krauss RM, Knoff LJ, et al. Modification of LCAT activity and HDL structure. New links between cigarette smoke and coronary heart disease risk. Arterioscler Thromb. 1994 Feb;14(2):248–53.
86. Halappanavar S, Stampfli MR, Berndt-Weis L, Williams A, Douglas GR, Yauk CL. Toxicogenomic analysis of mainstream tobacco smoke-exposed mice reveals repression of plasminogen activator inhibitor-1 gene in heart. Inhal Toxicol. 2009 Jan;21(1):78–85.
87. Gea J, Agustí A, Roca J. Pathophysiology of muscle dysfunction in COPD. J Appl Physiol. 2013 May;114(9):1222–34.
88. Prasad S, Sajja RK, Park JH, Naik P, Kaisar MA, Cucullo L. Impact of cigarette smoke extract and hyperglycemic conditions on blood-brain barrier endothelial cells. Fluids Barriers CNS. 2015 Jul 24;12:18.
89. Ohno N, Tanaka T, Kita T, Kubo K, Shimada K, Yonetani Y, et al. Changes of brain endothelin levels and peripheral endothelin receptors by chronic cigarette smoke in spontaneously hypertensive rats. J Pharmacol Sci. 2004 Mar;94(3):287–96.
90. Manna SK, Rangasamy T, Wise K, Sarkar S, Shishodia S, Biswal S, et al. Long term environmental tobacco smoke activates nuclear transcription factor-kappa B, activator protein-1, and stress responsive kinases in mouse brain. Biochem Pharmacol. 2006 May 28;71(11):1602–9.
91. Fuller BF, Gold MS, Wang KKW, Ottens AK. Effects of environmental tobacco smoke on adult rat brain biochemistry. J Mol Neurosci. 2010 May;41(1):165–71.
92. Madani A, Alack K, Richter MJ, Krüger K. Immune-regulating effects of exercise on cigarette smoke-induced inflammation. J Inflamm Res. 2018;11:155–67.
93. Pesch B, Kendzia B, Gustavsson P, Jöckel KH, Johnen G, Pohlabeln H, et al. Cigarette smoking and lung cancer--relative risk estimates for the major histological types from a pooled analysis of case-control studies. Int J Cancer. 2012 Sep 1;131(5):1210-9
94. Oh CK, Murray LA, Molfino NA. Smoking and idiopathic pulmonary fibrosis. Pulm Med. 2012; 2012: 808260
95. Bhat TA, Panzica L, Kalathil SG, Thanavala Y. Immune dysfunction in patients with chronic obstructive pulmonary disease. Ann Am Thorac Soc. 2015 Nov;12 Suppl 2:S169-175.
96. Neidich SD, Green WD, Rebeles J, Karlsson EA, Schultz-Cherry S, Noah TL, et al. Increased risk of influenza among vaccinated adults who are obese. Int J Obes (Lond). 2017 Sep;41(9):1324–30.
97. Macaulay R, Akbar AN, Henson SM. The role of the T cell in age-related inflammation. Age (Dordr). 2013 Jun;35(3):563–72.
98. Luo T, Nocon A, Fry J, Sherban A, Rui X, Jiang B, et al. AMPK Activation by metformin suppresses abnormal extracellular matrix remodeling in adipose tissue and ameliorates insulin resistance in obesity. Diabetes. 2016;65(8):2295–310.
99. Ohno H, Matsuzaka T, Tang N, Sharma R, Motomura K, Shimura T, et al. Transgenic mice overexpressing srebp-1a in male ob/ob mice exhibit lipodystrophy and exacerbate insulin resistance. Endocrinology. 2018 Jun 1;159(6):2308–23.
100. Park J-M, Chang K-H, Park K-H, Choi S-J, Lee K, Lee J-Y, et al. Differential effects between cigarette total particulate matter and cigarette smoke extract on blood and blood vessel. Toxicol Res. 2016 Oct;32(4):353–8.
101. Ponce-Gallegos MA, Ramírez-Venegas A, Falfán-Valencia R. Th17 profile in COPD exacerbations. Int J Chron Obstruct Pulmon Dis. 2017;12:1857–65.
102. Johnson AR, Milner JJ, Makowski L. The inflammation highway: metabolism accelerates inflammatory traffic in obesity. Immunol Rev. 2012 Sep;249(1):218–38.
103. Okla M, Zaher W, Alfayez M, Chung S. Inhibitory effects of toll-like receptor 4, NLRP3 inflammasome, and interleukin-1β on white adipocyte browning. Inflammation. 2018 Mar;41(2):626–42.
104. Samuvel DJ, Jin J, Sundararaj KP, Li Y, Zhang X, Lopes-Virella MF, et al. TLR4 activation and IL-6-mediated cross talk between adipocytes and mononuclear cells synergistically

stimulate MMP-1 expression. Endocrinology. 2011 Dec;152(12):4662–71.
105. Doz E, Noulin N, Boichot E, Guénon I, Fick L, Le Bert M, et al. Cigarette smoke-induced pulmonary inflammation is TLR4/MyD88 and IL-1R1/MyD88 signaling dependent. J Immunol. 2008 Jan 15;180(2):1169–78.
106. Kawasaki N, Asada R, Saito A, Kanemoto S, Imaizumi K. Obesity-induced endoplasmic reticulum stress causes chronic inflammation in adipose tissue. Sci Rep. 2012;2:799.
107. You T, Arsenis NC, Disanzo BL, Lamonte MJ. Effects of exercise training on chronic inflammation in obesity : current evidence and potential mechanisms. Sports Med. 2013 Apr;43(4):243–56.
108. Khadir A, Kavalakatt S, Cherian P, Warsame S, Abubaker JA, Dehbi M, et al. Physical exercise enhanced heat shock protein 60 expression and attenuated inflammation in the adipose tissue of human diabetic obese. Front Endocrinol (Lausanne). 2018;9:16.
109. Yakeu G, Butcher L, Isa S, Webb R, Roberts AW, Thomas AW, et al. Low-intensity exercise enhances expression of markers of alternative activation in circulating leukocytes: roles of PPARγ and Th2 cytokines. Atherosclerosis. 2010 Oct;212(2):668–73.
110. Barry JC, Simtchouk S, Durrer C, Jung ME, Little JP. Short-term exercise training alters leukocyte chemokine receptors in obese adults. Med Sci Sports Exerc. 2017 Aug;49(8):1631–40.
111. Shaw DM, Merien F, Braakhuis A, Dulson D. T-cells and their cytokine production: The anti-inflammatory and immunosuppressive effects of strenuous exercise. Cytokine. 2018 Apr;104:136–42.
112. Weinhold M, Shimabukuro-Vornhagen A, Franke A, Theurich S, Wahl P, Hallek M, et al. Physical exercise modulates the homeostasis of human regulatory T cells. J Allergy Clin Immunol. 2016;137(5):1607-1610.e8.
113. Rehm K, Sunesara I, Marshall GD. Increased circulating anti-inflammatory cells in marathon-trained runners. Int J Sports Med. 2015 Oct;36(10):832–6.
114. Kawanishi N, Mizokami T, Yano H, Suzuki K. Exercise attenuates M1 macrophages and CD8+ T cells in the adipose tissue of obese mice. Med Sci Sports Exerc. 2013 Sep;45(9):1684–93.
115. Kawanishi N, Yano H, Yokogawa Y, Suzuki K. Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. Exerc Immunol Rev. 2010;16:105–18.
116. Silveira LS, Batatinha HAP, Castoldi A, Câmara NOS, Festuccia WT, Souza CO, et al. Exercise rescues the immune response fine-tuned impaired by peroxisome proliferator-activated receptors γ deletion in macrophages. J Cell Physiol. 2018 Sep 21;
117. Rada I, Deldicque L, Francaux M, Zbinden-Foncea H. Toll like receptor expression induced by exercise in obesity and metabolic syndrome: A systematic review. Exerc Immunol Rev. 2018;24:60–71.
118. Robinson E, Durrer C, Simtchouk S, Jung ME, Bourne JE, Voth E, et al. Short-term high-intensity interval and moderate-intensity continuous training reduce leukocyte TLR4 in inactive adults at elevated risk of type 2 diabetes. J Appl Physiol. 2015 Sep 1;119(5):508–16.
119. Ringseis R, Eder K, Mooren FC, Krüger K. Metabolic signals and innate immune activation in obesity and exercise. Exerc Immunol Rev. 2015;21:58–68.
120. Campbell SC, Wisniewski PJ, Noji M, McGuinness LR, Häggblom MM, Lightfoot SA, et al. The Effect of Diet and Exercise on Intestinal Integrity and Microbial Diversity in Mice. PLoS One [Internet]. 2016 Mar 8 [cited 2018 Dec 10];11(3).
121. Pedersen BK, Steensberg A, Keller P, Keller C, Fischer C, Hiscock N, et al. Muscle-derived interleukin-6: lipolytic, anti-inflammatory and immune regulatory effects. Pflugers Arch. 2003 Apr;446(1):9–16.
122. Rinnov A, Yfanti C, Nielsen S, Akerström TCA, Peijs L, Zankari A, et al. Endurance training enhances skeletal muscle interleukin-15 in human male subjects. Endocrine. 2014 Mar;45(2):271–8.
123. Ye J. Beneficial metabolic activities of inflammatory cytokine interleukin 15 in obesity and type 2 diabetes. Front Med. 2015 Jun;9(2):139–45.
124. Kiniwa T, Enomoto Y, Terazawa N, Omi A, Miyata N, Ishiwata K, et al. NK cells activated by Interleukin-4 in cooperation with Interleukin-15 exhibit distinctive characteristics. Proc Natl Acad Sci USA. 2016 06;113(36):10139–44.
125. Saligrama PT, Fortner KA, Secinaro MA, Collins CC, Russell JQ, Budd RC. IL-15 maintains T-cell survival via S-nitrosylation-mediated inhibition of caspase-3. Cell Death Differ. 2014 Jun;21(6):904–14.
126. Stockley RA, Mannino D, Barnes PJ. Burden and pathogenesis of chronic obstructive pulmonary disease. Proc Am Thorac Soc. 2009 Sep 15;6(6):524–6.
127. Kantorowski A, Wan ES, Homsy D, Kadri R, Richardson CR, Moy ML. Determinants and outcomes of change in physical activity in COPD. ERJ Open Res. 2018 Jul;4(3).
128. Petersen AMW, Mittendorfer B, Magkos F, Iversen M, Pedersen BK. Physical activity counteracts increased whole-body protein breakdown in chronic obstructive pulmonary disease patients. Scand J Med Sci Sports. 2008 Oct;18(5):557–64.
129. Menegali BT, Nesi RT, Souza PS, Silva LA, Silveira PCL, Valença SS, et al. The effects of physical exercise on the cigarette smoke-induced pulmonary oxidative response. Pulm Pharmacol Ther. 2009 Dec;22(6):567–73.
130. Onur E, Kabaroğlu C, Günay O, Var A, Yilmaz O, Dündar P, et al. The beneficial effects of physical exercise on antioxidant status in asthmatic children. Allergol Immunopathol (Madr). 2011 Apr;39(2):90–5.
131. Yu Y-B, Liao Y-W, Su K-H, Chang T-M, Shyue S-K, Kou YR, et al. Prior exercise training alleviates the lung inflammation induced by subsequent exposure to environmental cigarette smoke. Acta Physiol (Oxf). 2012 Aug;205(4):532–40.
132. Bowen TS, Aakerøy L, Eisenkolb S, Kunth P, Bakkerud F, Wohlwend M, et al. Exercise training reverses extrapulmonary impairments in smoke-exposed mice. Med Sci Sports Exerc. 2017;49(5):879–87.
133. Gibala MJ, Little JP, Macdonald MJ, Hawley JA. Physiological adaptations to low-volume, high-intensity interval training in health and disease. J Physiol (Lond). 2012 Mar 1;590(5):1077–84.
134. Cugno M, Agostoni P, Mari D, Meroni PL, Gregorini L, Bussotti M, et al. Impaired bradykinin response to ischaemia and exercise in patients with mild congestive heart failure during angiotensin-converting enzyme treatment. Relationships with endothelial function, coagulation and inflammation. Br J Haematol. 2005 Jul;130(1):113–20.

135. Spruit MA, Burtin C, De Boever P, Langer D, Vogiatzis I, Wouters EFM, et al. COPD and exercise: does it make a difference? Breathe (Sheff). 2016 Jun;12(2):e38-49.
136. Spruit MA, Wouters EFM. New modalities of pulmonary rehabilitation in patients with chronic obstructive pulmonary disease. Sports Med. 2007;37(6):501–18.
137. Komine S, Akiyama K, Warabi E, Oh S, Kuga K, Ishige K, et al. Exercise training enhances in vivo clearance of endotoxin and attenuates inflammatory responses by potentiating Kupffer cell phagocytosis. Sci Rep. 2017 Sep 20;7(1):11977.
138. Kasawara KT, Cotechini T, Macdonald-Goodfellow SK, Surita FG, Pinto E Silva JL, Tayade C, et al. Moderate exercise attenuates lipopolysaccharide-induced inflammation and associated maternal and fetal morbidities in pregnant rats. PLoS ONE. 2016;11(4):e0154405.
139. Lee J, Lee Y, LaVoy EC, Umetani M, Hong J, Park Y. Physical activity protects NLRP3 inflammasome-associated coronary vascular dysfunction in obese mice. Physiol Rep. 2018 Jun;6(12):e13738.
140. Shimobayashi M, Albert V, Woelnerhanssen B, Frei IC, Weissenberger D, Meyer-Gerspach AC, et al. Insulin resistance causes inflammation in adipose tissue. J Clin Invest. 2018 Apr 2;128(4):1538–50.
141. Ok D-P, Ko K, Bae JY. Exercise without dietary changes alleviates nonalcoholic fatty liver disease without weight loss benefits. Lipids Health Dis. 2018 Sep 1;17(1):207.
142. Biswas A, Oh PI, Faulkner GE, Bajaj RR, Silver MA, Mitchell MS, et al. Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults: a systematic review and meta-analysis. Ann Intern Med. 2015 Jan 20;162(2):123–32.
143. Anstey KJ, Kingston A, Kiely KM, Luszcz MA, Mitchell P, Jagger C. The influence of smoking, sedentary lifestyle and obesity on cognitive impairment-free life expectancy. Int J Epidemiol. 2014 Dec;43(6):1874–83.
144. Barboza ML, Barbosa ACB, Spina GD, Sperandio EF, Arantes RL, Gagliardi AR de T, et al. Association between physical activity in daily life and pulmonary function in adult smokers. J Bras Pneumol. 2016 Apr;42(2):130–5.
145. Fiuza-Luces C, Santos-Lozano A, Joyner M, Carrera-Bastos P, Picazo O, Zugaza JL, et al. Exercise benefits in cardiovascular disease: beyond attenuation of traditional risk factors. Nat Rev Cardiol. 2018 Aug 16;

Hallmarks of Improved Immunological Responses in the Vaccination of More Physically Active Elderly Females

Glenn Choon Lim Wong[1], Vipin Narang[1], Yanxia Lu[1], Xavier Camous[1], Ma Shwe Zin Nyunt[2], Christophe Carre[3], Crystal Tan[1], Chin Hui Xian[1], Joni Chong[1], Michelle Chua[1], Wilson How[1], Esther Mok[1], Paul Tambyah[4], Michael Poidinger[1], Brian Abel[1], Nicolas Burdin[3], Laurence Quemeneur[3], Nabil Bosco[5], Tze Pin Ng[2], Anis Larbi[1]

[1] Singapore Immunology Network (SIgN), Agency for Science Technology and Research (A*STAR), Immunos, Singapore
[2] Department of Psychological Medicine, Yong Loo Lin School of Medicine, National University of Singapore
[3] Sanofi Pasteur, Marcy l'Etoile, France
[4] Division of Infectious Diseases, National University Hospital, Singapore.
[5] Nestlé Research Centre (Singapore), 21 Biopolis Road, Singapore

ABSTRACT

Physical inactivity is one of the leading contributors to worldwide morbidity and mortality. The elderly are particularly susceptible since the features of physical inactivity overlap with the outcomes of natural aging – including the propensity to develop cardiovascular diseases, cancer, diabetes mellitus, sarcopenia and cognitive impairment. The age-dependent loss of immune function, or immunosenescence, refers to the progressive depletion of primary immune resources and is linked to the development of many of these conditions. Immunosenescence is primarily driven by chronic immune activation and physical activity interventions have demonstrated the potential to reduce the risk of complications in the elderly by modulating inflammation and augmenting the immune system. Since poor vaccination outcome is a hallmark of immunosenescence, the assessment of vaccine efficacy provides a window to study the immunological effects of regular physical activity. Using an accelerator-based study, we demonstrate in a Singaporean Chinese cohort that elderly women (n=56) who walk more after vaccination display greater post-vaccination expansion of monocytes and plasmablasts in peripheral blood. Active elderly female participants also demonstrated lower baseline levels of IP-10 and Eotaxin, and the upregulation of genes associated with monocyte/macrophage phagocytosis. We further describe postive correlations between the monocyte response and the post-vaccination H1N1 HAI titres of participants. Finally, active elderly women reveal a higher induction of antibodies against Flu B in their 18-month second vaccination follow-up. Altogether, our data are consistent with better immunological outcomes in those who are more physically active and highlight the pertinent contribution of monocyte activity.

Keywords: Aging, Physical Activity, Influenza, Vaccination, Immunosenescence

Corresponding Author:
Anis Larbi, anis_larbi@immunol.a-star.edu.sg. Biology of Aging Laboratory, Singapore Immunology Network, 8A Biomedical Grove, Level 3 & 4, Immunos Building, Singapore 138648.

1. Introduction

Physical inactivity has been cited by the WHO as the fourth leading risk factor for global mortality (6% of deaths globally) – preceded only by high blood pressure (13%), tobacco use (9%), and high blood glucose (6%) (80). Physical activity, defined as bodily movement directed by the skeletal muscles and resulting in energy expenditure, differs from exercise as the latter refers to planned, structured, and repetitive programmes that are designed to maintain and improve physical fitness (10). Both physical activity and exercise are positively associated with beneficial outcomes in cardiorespiratory (CHD, CVD, stroke, hypertension), muscular (muscle mass, strength and power), metabolic (diabetes, metabolic syndrome), mental (depression) and immunological (immune activation levels, vaccination efficacy and immunosenescence) health (28, 29, 32, 33, 46, 60, 67, 74). Since the elderly are susceptible to a decline in all these faculties, physical activity becomes an attractive prospect of a low-cost intervention, particularly since pharmacological interventions have led to more controversial or limited efficacy in delaying the acquisition of age-related morbidities (6).

The world is aging rapidly and the population of elderly over the age of 65 is expected to approach 1.5 billion by the year 2050 (79). In alleviating the growing demands on healthcare that accompany the aging phenomenon – the elderly impose a disproportionate reliance on these resources – governments must introduce policies that keep the elderly robust. While the benefits of physical activity are relevant throughout lifespan, there is a growing body of literature that describe more pertinent benefits of physical activity in the elderly (14, 75). The age-related loss of immune function is particularly well documented as a driver of age-related causes of morbidity and mortality; the former is widely attributed to the concomitant loss of hematopoietic resources and naïve lymphocytes as well as an overrepresentation of late-stage memory lymphocytes – altogether constituting an immune system that is less poised to respond to both old and new antigens (39). Although this scenario results in poor vaccination outcomes in the elderly, there is compelling evidence that physical activity levels can compensate for these deficiencies (14, 59, 75).

Differences in population demographics or the nature of physical activity have however, contributed to heterogeneous

observations among studies that investigate the modulatory effect of physical activity on vaccination outcomes. Perhaps due to age or uniformed high levels of vaccine-responsiveness, a study on young college students demonstrated that neither physical fitness nor physical activity improved antibody responses after influenza vaccination (70). A separate study found that the antibody response of elite swimmers (aged 17-23) to pneumococcal vaccination encompassed more antibody subtypes than their sedentary counterparts (29). Nevertheless, the impact of physical activity or exercise on vaccination responses in the elderly has generally been described as positive. In the elderly, the augmentation of immune responses by physical activity has been demonstrated in the contexts of pneumococcal, meningococcal and influenza vaccinations (35, 36, 60, 69). The latter may be linked to better immunosenescent profiles in the elderly, since independent studies have revealed reduced frequencies of senescent naïve and memory T-cells as well as a reduced inflammatory signature in those who exercise regularly (46, 52, 73). Unfortunately, studies linking physical activity to advantageous influenza vaccination responses rarely investigate beyond HAI titres. Without the elaborate characterisation of post-vaccination immunity, it is a challenge to determine the nature of physical activity that is required to optimise these benefits.

In summary, while there is a strong case for promoting physical activity as a fundamental component to healthy aging, the results of these studies display a heavy reliance on self-reported physical activity or organised exercise regimes – which may be challenging to interpret and implement on a national or global level (60, 74). Moreover, the specific mechanisms responsible for generating these effects remain elusive, for example, it is unclear whether the enhancement of post-vaccination immunity results from changes in the innate or adaptive immune systems. In this study, we aim to study the value and impact of physical activity on post-vaccination immunity using a more comprehensive and multi-dimensional approach; we also validate the application of an accelerometer in achieving these aims. Using data from an accelerometer-based study of an elderly Singaporean Chinese cohort, we demonstrate that elderly women who walk more display greater post-vaccination expansion of monocytes and plasmablasts in peripheral blood compared with sedentary women. Through the transcriptomic analysis of PBMC-derived mRNA, we further reveal the upregulation of genes associated with monocyte/macrophage phagocytosis in active elderly women. Importantly, we found significant correlations between the post-vaccination expansion of monocytes and H1N1 HAI titres. Finally, active elderly women who walk more mount a stronger antibody response against Flu B in their 18-month second vaccination follow-up. Altogether, our data are consistent with enhanced vaccination outcomes in physically active females, mediated by both the innate and adaptive immune system.

2. Materials and Methods

Study participants

The National Healthcare Group (NHG) Domain Specific Institutional Review Board (DSRB) approved a phase IV clinical trial of Sanofi Pasteur's Vaxigrip© influenza vaccine, which is registered at clinicaltrials.gov under the registration number NCT03266237. We recruited older adults above 65 years of age for participation in an epidemiological study of a second cohort from the Singapore Longitudinal Aging Study (SLAS-2) (51). Participants were community dwellers from eight different housing precincts across Singapore. Volunteers were excluded if they had received an influenza vaccination in the past 6-months. Those with suspected congenital or acquired immunodeficiency as well as those receiving immunosuppressive or long-term corticosteroid therapy were also excluded. All volunteers provided written informed consent for the reception of Vaxigrip©.

Influenza vaccination

Two commercially available inactivated trivalent Vaxigrip© (2013-14) seasonal influenza vaccines were used in this trial. In the first vaccination, vaccine consisted of split virions from three prevalent strains: A/California/07/2009 (H1N1), A/Texas/50/2012 (H3N2) and B/Massachusetts/02/2012. For the second vaccination at the 20-month follow-up, this was updated to A/California/07/2009 (H1N1), A/Switzerland/97/2013 (H3N2) and B/Phuket/3073/2013. Virions were grown in embryonated chicken eggs, inactivated by formaldehyde treatment, and split via anionic detergent. The first vaccine was administered to elderly study participants between January and August 2014. Venous blood specimens were collected from the participants immediately prior to vaccination (Day 0) and on Days 2, 7 and 28 after vaccination. For the second vaccination, blood specimens were collected immediately prior to vaccination and 28-days post-vaccination. Participants with HAI titres >40 (1/dil) were considered seroprotected.

Actigraphy data collection

On the day of vaccination, participants were given a wrist-worn device, Actical© (Phillips, Amsterdam, Netherlands), which they agreed to wear on their wrist for a 14-day period that commenced immediately after vaccination. The device does not interfere with any of the participant's daily activities and participants were not instructed to modify their daily routine and behaviour. The device was used to monitor gross motor activity and collected data were analysed by the software Actical© v3.1 (Philips Electronics, Amsterdam, Netherlands), which in addition to the number of steps completed, provides percentage data on the participant's sedentary period. Although 183 participants consented to actigraphy data collection, we divided participants into quartiles based on their number of steps completed. Participants who belonged to the highest and lowest quartiles were included in the analyses described in this study (Females, n=56; Males, n=34).

Determination of Frailty Status

At the time of recruitment, demographic, medical, psychosocial, behavioural, and neurocognitive variables were collected from study participants by clinical personnel through interviews and on-site clinical assessment. Data specific to the Instrumental Activities of Daily Living (IADL), Short-from Health Survey (SF-12), Mini Mental State Examination (MMSE), Geriatric Depression Scale (GDS), Geriatric Anxiety Inventory (GAI) and Montreal Cognitive Assessment (MOCA) were collected as described in validated studies (40,

48, 58, 59, 77, 82). In addition, participants self-reported answers to questions related to their occupational, socio-economic, medical history, exercise and nutritional habits, which were presented in the form of a questionnaire. Physical frailty was defined according to Fried's assessment of five criteria, including unintentional weight loss, slowness, weakness, exhaustion, and low physical activity (25), and scaled to Asian populations as described previously (51).

Characterisation of Sarcopenic Status

Total and regional lean body mass was measured by dual energy X-ray absorptiometry (DXA) scan with a Hologic® densitometer. The participant laid in a supine position on the DXA table with limbs close to the body. DXA scans were performed in the Department of Diagnostic Radiology, National University Hospital (NUH) of Singapore. Appendicular muscle mass was calculated as described by Heymsfield *et. al.* (31). Lower limb strength was assessed using the methods described by Lord *et. al.,* and body mass index (BMI) values were calculated by averaging triplicate measurements (45). Participants were required to complete the 6-meter gait speed test as described by Nelson *et al.,* duplicate measurements were averaged for the determination of gait speed in meters/second (50). In accordance to recommendations by the Asian Working Group for Sarcopenia (AWGS), sarcopenic status was determined using measures of appendicular lean mass, lower leg limb strength and gait speed (11). The appendicular skeletal muscle index (ASMI) was calculated as the ratio between appendicular muscle mass and height^2. Low ASMI refers to values below 7.0 kg/m^2 and 5.4 kg/m^2 for men and women respectively. Cut-off values for low lower limb strength are as indicated: ≤ 18 kg for men and ≤ 16 kg for women. Low gait speed was defined as an average speed of 2 trials ≤ 0.8 m/s. Finally, a participant was categorized as sarcopenic if they had low ASMI and low lower limb strength and/or gait speed.

Vaccine-specific (HAI) antibody titres

Vaccine-specific antibodies were measured using sera collected at Days 0 and 28 after both vaccinations using the Hemagglutination inhibition (HAI) assay. Two independent assays were concluded for the determination of HAI titres. Samples were first heat inactivated and pre-treated with neuraminidase to eliminate non-specific inhibitors and anti-turkey red blood cell (anti-TRBC) hemagglutinins, which may interfere with the test results. Treated sera were then serially titrated, starting at a 1/10 dilution, and incubated with 4 hemagglutinating units/25 µl of the virus. After incubation at 37°C for 1 h, a 0.5% TRBC suspension was added to the plates and was incubated for one hour at ambient temperature. Plates were then read using the tilt method, and the HAI titre was assigned as the reciprocal of the highest serum dilution that exhibited complete inhibition of hemagglutination. The geometric mean titre of both independent runs was used to determine the final titre.

Cytokine Measurements by Luminex

The Milliplex© human cytokine/chemokine panel (Cat: EPX090-12187-901; Merck Millipore, Massachusetts, USA) was used to measure the levels of IFNγ-inducible protein 10 (IP-10) in plasma samples from participants that were collected at baseline and Days 2, 7 and 28 post-vaccination. Eotaxin and high sensitivity C-reactive protein (hs-CRP) were only measured at baseline. Samples and standards were incubated with fluorescent-coded magnetic beads which had been pre-coated with the respective capture antibodies. After overnight incubation at 4°C with shaking, plates were washed twice with wash buffer. Biotinylated detection antibodies were incubated with the complex for 1 hour and Streptavidin-PE was subsequently added for another 30 mins incubation. Plates were washed twice before beads were re-suspended in sheath fluid and read using PCR plates by the Luminex analyser, FLEXMAP© 3D (Merck Millipore, Massachusetts, USA). Data were acquired using the xPONENT© 4.0 (Luminex©, Texas, USA) acquisition software and analysed with a Bio-Plex Manager 6.1.1© (Bio-Rad, California, USA). Standard curves were generated, and a 5-parameter logistic algorithm were used for the estimation of MFI and concentration values.

Immunophenotyping by flow cytometry

Percentages of immune cell subsets were determined using cryopreserved peripheral blood mononuclear cells (PBMCs). Cryopreserved PBMCs were quick-thawed at 37˚C and resuspended in warm RPMI. After a second wash, PBMCs were counted and plated into 96-well plates for subsequent stain-

Table I: T-cell Antibody Panel

Target	Company	Clone	Dye	Catalog
Vd1	Abcam	TS8.2	FITC	ab171097
Vd2	Biolegend	B6	PerCP	331410
CD4	Biolegend	OKT4	PE-Cy5	317412
CD31	Biolegend	WM59	BV605	303122
CTLA4	Biolegend	L3D10	PE-Cy7	349914
CXCR5	Biolegend	J252D4	PE	356904
PD1	eBioscience	eBioJ105	APC-eF780	47-2799-42
CD3	BD	UCHT1	AF700	557943
CD8	BD	RPA-T8	BV711	563677
CD27	BD	L128	BV650	563228
CD28	BD	CD28.2	BUV737	564438
CD45RO	BD	UCHL1	BUV 395	564291
CD95	BD	DX2	PE-CF594	562395
KLRG1	BD	2F1/KLRG1	APC	561620

Table II: B-cell Antibody Panel

Target	Company	Clone	Dye	Catalog
CD10	BD	HI10a	PE-CF594	562396
CD19	BD	HIB19	AF700	557921
CD21	BD	B-ly4	PE-Cy5	551064
CD24	BD	ML5	BV421	562789
CD27	BD	L128	BV650	563228
CD38	BD	HIT2	APC	555462
CD45	BD	HI30	PerCP/Cy5.5	564105
CD138	BD	MI15	BV605	563294
IgD	BD	IA6-2	FITC	555778
IgM	Biolegend	MHM-88	APC-Cy7	314520
CD20	Biolegend	2H7	BV570	302332
CD40	Biolegend	5C3	PE-Cy7	334322
CXCR5	Biolegend	J252D4	PE	356904
HLA-DR	Biolegend	L243	BV785	307642

ing. 0.5, 0.75 and 1.5 million cells were used for T-cell, B-cell and Myeloid phenotyping panels respectively (Tables I, II and III respectively); antibodies were procured from eBioscience (California, USA), Biolegend (California, USA), BD Biosciences (California, USA) and Miltenyi Biotec (Bergisch Gladback, Germany). All panels contain a Thermo Fisher Live/Dead© fixable dye (Cat: L-34957; Thermo Fisher, Massachusetts, USA) to distinguish between live and dead cells. Antibody cocktails were added to aliquoted cells and incubated for 20 minutes in the dark at 4˚C. The cells were then washed twice before resuspension in staining buffer and analysed on the LSR II (BD Biosciences, California, USA) cytometer. Data generated by flow cytometry was analysed with the Flowjo© software (Tree Star, Inc., USA). Events were gated by forward and side scatter followed by subset-specific marker expression.

For deriving absolute counts, the numbers of monocytes, B cells, plasmablasts, CD4 and CD8 T cells, NK cells, and conventional and plasmacytoid dendritic cells were determined using freshly collected whole blood samples. 100 µL of whole blood was stained with antibody cocktail (Table IV) in BD Trucount© Absolute Counting Tubes (Cat: 340334; BD Biosciences, California, USA) for 15 minutes at room temperature. 900 µL of 1X BD FACS Lysing solution (Cat: 349202; BD Biosciences, California, USA) was then added to the tube and incubated for 15 minutes before acquisition on the LSR II Fortessa (BD Biosciences, California, USA) flow cytometer.

Table III: Myeloid Antibody Panel

Target	Company	Clone	Dye	Catalog
CD3 (Lin)	BD	UCHT1	AF700	557943
CD19 (Lin)	BD	H1B19	AF700	557921
CD20 (Lin)	Biolegend	2H7	AF700	302322
CD56 (Lin)	Biolegend	HCD56	AF700	318316
CD2	BD	RPA-2.10	APC H7	562638
CD11c	BD	BLy6	FITC	561355
CD15	BD	HI98	BUV395	563872
CD16	BD	3G8	BUV 737	564434
CD33	BD	WM53	BV421	562854
CD45	BD	HI30	PerCP-Cy5.5	564105
CD80	BD	L307.4	BV650	564158
CD1c	Biolegend	L161	PE-Cy7	331516
CD11b	Biolegend	ICRF44	BV711	301344
CD14	Biolegend	HCD14	PE-Dazzle 594	325634
CD123	Biolegend	6H6	BV510	306022
CD141	Biolegend	M80	APC	344106
HLA DR	Biolegend	L243	BV605	307640
SLAN	Miltenyi	DD-1	PE	130-093-029

Table IV: Trucount Antibody Panel

Target	Company	Clone	Dye	Catalog
CD19	BD	SJ25C1	BV786	563325
CD38	BD	HIT2	APC	555462
CD3	Biolegend	OKT3	PE/cy7	317334
CD4	Biolegend	OKT4	BV510	317444
CD14	Biolegend	M5E2	PerCP	301824
CD16	Biolegend	3G8	A700	302026
CD27	Biolegend	M-T271	PE	356406
CD45	Biolegend	HI30	PB	304029
CD62L	Biolegend	DREG-56	APC/cy7	304814
CD66b	Biolegend	G10F5	FITC	305104
HLA-DR	Biolegend	L243	BV605	307640
CD8	eBioscience	RPA-T8	PE TR	61-0088-42
CD56	eBioscience	CMSSB	PE/cy5.5	35-0567-42
CD123	eBioscience	6H6	BV650	95-1239-42

Determination of Vaccine Specific B-cells by ELISpot

PBMCs were thawed and counted as described in the immunophenotyping section. PBMCs were plated into 4 wells at 0.1 million PBMCs per well, incubated with IL-21, and separately treated with the following conditions: unstimulated, vaccine-stimulated (0.3 µg/mL of trivalent vaccine) in duplicates and positive control as stimulated by both anti-CD40 (Cat: MAB6321; RnD Systems, Minnesota, USA) and CpG oligonucleotides (CpG ODN 2006, Cat: tlrl-2006; Invivogen, California, USA). PBMCs were stimulated for 22 hours in a humidified 37˚C CO2 incubator. Vaccine-specific IgG-secreting B-cells were detected using the Human IgG ELISpot kit© (Cat: 3850-2H; Mabtech, Nacka Strand, Sweden) by adhering to the manufacturer's protocol. ELISpot plates were read using an ImmunoSpot S5 Versa Analyser (Cellular Technology Limited, Ohio, USA). The enumeration of vaccine-specific IgG-secreting B-cells was performed by averaging the number of spots detected from duplicate vaccine-stimulated wells.

Microarray, Differentially Expressed Genes (DEGs) and Ingenuity Pathway Analysis (IPA)

Total ribonucleic acid (RNA) were isolated from PBMCs using the mirVana© miRNA isolation kit (Cat: AM1560; Thermo Fisher Scientific, California, USA). Complementary DNA (cDNA) were generated and purified using the cDNA synthesis kit (Cat: K2561; Thermo Fisher Scientific, California, USA). Gene expression was analysed by microarray using the Illumina human HT-12 V4.0 bead chip platform, TargetAmp©-Nano-g Biotin-aRNA Labeling Kit for Illumina (Cat: TAN07908; Epicenter, Wisconsin, USA). The raw gene expression data output from Illumina Genome Studio© (Illumina, California, USA) were exported in batches of 96 samples (21). Quality control (QC) and pre-processing of data were completed using Bioconductor© packages for the R software. The data were log2 transformed and normalized using the Robust Spline Normalization (RSN) method available in the Lumi package (Bioconductor) (17). Only probes which passed a detection p-value of 0.05 in 90% of the subjects were retained. Differential gene expression analysis between sedentary and active individuals was performed using the Limma package (Bioconductor) with age included as a cofactor within the linear model (68). Differentially expressed genes (DEGs) were selected based on a nominal p-value < 0.05. The list of DEGs along with expression fold changes and p-values were loaded into Ingenuity Pathway Analysis software (IPA®; Qiagen Bioinformatics, California, USA). A standard core analysis was performed in IPA to identify pathways enriched with the DEGs (38). The microarray data is available on Gene Expression Omnibus (NCBI) under the accession number GSE107990 (13).

Statistical Methods

Demographic parameters were compared between sedentary and active individuals by single-factor ANOVA for numerical and Fisher's exact test for categorical data, respectively. Per-

Table 1: Participation Demographics and Serology

	Female			Male		
Participant Demographics	Sedentary (n = 28)	Active (n = 28)	p-value	Sedentary (n = 17)	Active (n = 17)	p-value
Age	75.4 ± 5.1	70.2 ± 3.9	0.00	75.9 ± 3.9	72.1 ± 4.5	0.01
Frail	13 (46%)	10 (36%)	0.59	8 (47%)	5 (29%)	0.48
Frailty Score	2.2 ± 1.5	1.8 ± 1.5	0.37	1.9 ± 1.3	1.6 ± 1.2	0.42
Sarcopenia	10 (36%)	13 (46%)	0.59	7 (42%)	3 (18%)	0.05
BMI	23.1 ± 2.3	22.9 ± 3.5	0.77	23.4 ± 3.8	24.2 ± 2.3	0.43
Waist circumference (cm)	84.1 ± 11.2	81.1 ± 6.5	0.24	90.7 ± 12.00	89.5 ± 6.00	0.73
Smoker (Past or Present)	0 (0%)	1 (4%)	1.00	9 (53%)	7 (41%)	0.73
Currently employed	0 (0%)	6 (21%)	0.02	1 (6%)	2 (12%)	0.60
Number of Comorbidities	2.2 ± 1.5	1.7 ± 1.4	0.77	1.9 ± 1.3	1.4 ± 1.2	0.43
High Blood Pressure	14 (50%)	12 (43%)	0.79	12 (71%)	10 (59%)	0.26
High Cholesterol	18 (64%)	14 (50%)	0.42	10 (59%)	9 (53%)	1.00
Diabetes	8 (29%)	5 (18%)	0.55	5 (29%)	4 (24%)	0.63
Pre-diabetic (abnormal fasting glucose)	11 (39%)	12 (43%)	1.00	4 (24%)	6 (35%)	0.71
Arthritis	7 (25%)	4 (14%)	0.50	4 (24%)	0 (0%)	0.10
Undergone Surgery	19 (68%)	17 (61%)	0.78	6 (35%)	6 (35%)	1.00
Mini Mental State Examination (MMSE)	26.5 ± 2.4	27.8 ± 2.4	0.05	25.4 ± 5.0	28.2 ± 1.4	0.03
Montreal Cognitive Assessment (MoCA)	24.6 ± 4.1	25.9 ± 3.00	0.24	23.4 ± 4.8	25.8 ± 1.9	0.08
Geriatric Anxiety Inventory (GAI)	0.3 ± 1.0	0.4 ± 1.3	0.73	0.5 ± 1.2	0.0 ± 0.0	0.11
Geriatric Depression Score (GDS)	0.8 ± 0.9	1.0 ± 1.5	0.45	0.76 ± 1.7	0.3 ± 0.9	0.32
Immunity and Post-Vaccine Seroprotection						
CD4 T cells (cells/ul)	673.5 ± 232.3	682.8 ± 270.4	0.9	617.9 ± 220.6	763.8 ± 598.2	0.35
CD8 T cells (cells/ul)	307.1 ± 151.8	331.0 ± 221.3	0.64	392.2 ± 272.7	432.8 ± 365.9	0.72
CD4:CD8 Ratio	2.6 ± 1.2	2.8 ± 2.3	0.66	2.0 ± 1.2	2.2 ± 1.4	0.63
Seroprotection H1N1	27 (96%)	27 (96%)	1.00	15 (88%)	14 (82%)	1.00
Seroprotection H3N2	28 (100%)	27 (96%)	1.00	15 (88%)	15 (88%)	1.00
Seroprotection B	28 (100%)	28 (100%)	1.00	17 (100%)	15 (88%)	0.49

Demographic parameters were compared between sedentary and active females (n=56) and males (n=34) by single-factor ANOVA and Fisher's exact test for numerical and categorical data respectively.

Table 2. DEXA indices of active and sedentary participants

Female	Sedentary (n = 28)	Active (n = 28)	p-value
L arm BMD (g/cm²)	0.56 ± 0.07	0.60 ± 0.04	0.01
R arm BMD (g/cm²)	0.58 ± 0.06	0.62 ± 0.05	0.02
L arm BMC (g)	83.00 ± 21.44	93.56 ± 17.87	0.05
R ribs Area (cm²)	129.78 ± 25.86	118.26 ± 18.25	0.06
T spine Area (cm²)	113.88 ± 20.71	123.02 ± 14.37	0.06
Neck BMD (g/cm²)	0.55 ± 0.08	0.59 ± 0.09	0.07
Total % body fat age matched	35.29 ± 25.66	24.74 ± 18.22	0.10
Trunk Fat mass (g)	11525.37 ± 3609.52	10220.56 ± 2114.92	0.11
Neck BMC (g)	2.49 ± 0.37	2.66 ± 0.39	0.11
Total BMC (g)	1481.1 ± 283.18	1609.95 ± 313.26	0.11
Male	**Sedentary (n = 17)**	**Active (n = 17)**	**p-value**
R arm Lean + BMC (g)	2488.85 ± 339.51	2984.23 ± 241.58	0.00
Gynoid % Fat	33.59 ± 4.78	28.62 ± 3.3	0.00
Gynoid Lean + BMC (g)	5966.62 ± 737.74	6766.08 ± 679.93	0.00
Appendicular Lean/height² (kg/m²)	6.24 ± 0.82	7.03 ± 0.51	0.01
L arm Lean + BMC (g)	2150.62 ± 365.14	2477 ± 195.57	0.01
R ribs BMD (g/cm2)	0.56 ± 0.08	0.63 ± 0.06	0.02
Lean/height2 (kg/m2)	14.64 ± 1.71	16.12 ± 1.05	0.02
R leg % Fat	30.83 ± 5.38	26.08 ± 4.73	0.03
R arm BMD (g/cm²)	0.71 ± 0.08	0.78 ± 0.06	0.03
R arm % Fat	31.9 ± 6.78	25.95 ± 6.25	0.03
R arm BMC (g)	156.04 ± 28.16	177.63 ± 17.80	0.03
Average legs %fat	30.58 ± 5.57	25.97 ± 4.74	0.03
Pelvis BMC (g)	155.93 ± 41.87	190.34 ± 39.43	0.04
L leg % Fat	30.34 ± 5.81	25.85 ± 4.77	0.04
Average arms % Fat	32.12 ± 6.55	27.06 ± 5.69	0.05
Subtotal Lean + BMC (g)	38494.85 ± 4374.94	41609.85 ± 3141.58	0.05

Demographic parameters were compared between sedentary and active individuals males (n= 34) and females (n=56) by single-factor ANOVA.

centages of immune cell subsets measured by flow cytometry were compared between sedentary and active individuals using an ANCOVA (analysis of covariance) test with age listed as a covariate. Comparisons across multiple time points within the same set of individuals were performed using the paired Mann-Whitney-Wilcoxon U test. HAI titres and blood counts of immune cells were log2 transformed. Pearson's correlation coefficient between these measurements was calculated across both sedentary and active subjects. P-values of correlation were computed using Pearson's product moment correlation coefficient test in R statistical language using the function cor.test.

3. RESULTS

3.1 Participant demographics and cohort description

A cohort of 183 elderly participants agreed to carry an Actical© (Phillips) device on their non-dominant wrists for a period of 14 days following vaccination with Vaxigrip©. The device records both actigraphy data as well as the number of steps completed by each participant. We divided elderly participants into quartiles based on the number of steps they completed (Females (n=112): LQ <10927 steps/day (n=28), HQ >18509 steps/day (n=28); Males (n=70): LQ <7174 steps/day (n=17), HQ >14770 steps/day (n=17) to group participants into either sedentary or physically active subgroups for further analysis. As the age difference between high and low activity groups was significant across both genders, we adjusted for age in all subsequent analyses. Although the initial plan was to compare individuals from the lowest (sedentary) and highest quartiles (active) in a gender-independent manner, we found that elderly females generally completed more steps than their male counterparts (Figure 1A). Furthermore, the distribution of steps completed among individuals in the highest quartile was more continuous for females than for males. In addition, stratification by sarcopenic status revealed a difference between high activity and low activity males (Table 1). Concordantly, as revealed by Dual-energy X-ray absorptiometry (DEXA) results, active males were more physically robust than their more sedentary counterparts – the most significant differences between active and sedentary males and females are listed in Table 2. Elderly males who walked more had higher bone mineral density (BMD) and content (BMC) in their arms, legs, pelvis and ribs; and lower fat content in their leg and gynoid regions. Although active females displayed trends of higher BMD and BMC, as well as lower fat composition, these differences were statistically

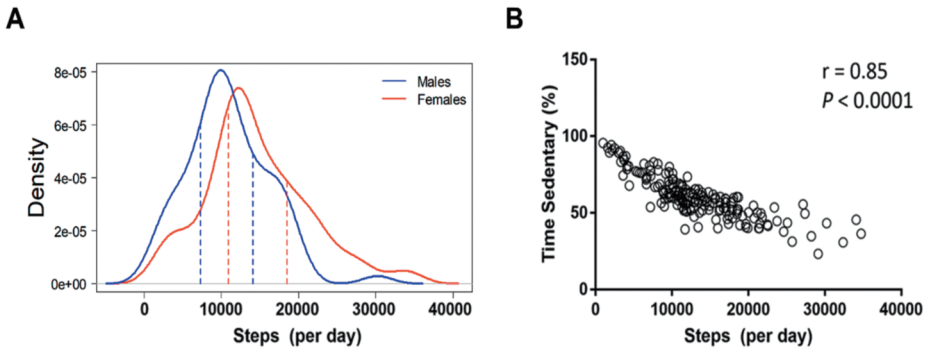


Figure 1. Baseline demographics of active and sedentary elderly participants. (A) Distribution of steps for all male (blue) and female (red) participants in the actigraphy study (n=183). Lines demarcate the boundaries of the interquartile range. (B) Correlation between percentage of sedentary time and the number of steps completed per day for all participants (n=183).

significant only for arm BMD (P < 0.02). The number of steps completed by all participants (n=183) was also well correlated to the sedentary period for each subject (Figure 1B), suggesting that both parameters are closely linked in this study (r = 0.85, P < 0.0001).

Possibly related to differences in ethnicity and infection history, we achieved relatively high post-vaccination seroprotection rates in our cohort of community dwelling elderly subjects (> 96% for females; > 82% in males). We observed no differences in the presence of comorbidities (high blood pressure, high cholesterol, diabetic status, arthritis) between active and sedentary participants. As reflected by their mini mental state examination (MMSE) scores, cognitive functionality was improved for elderly who were more physically active (p = 0.05 for females; p = 0.032 for males), although this difference was not statistically significant when participants were evaluated by their Montreal cognitive assessment (MoCA) scores. Due to fewer male participants (n=34), the lack of a distinct population of highly active male individuals and the large number of confounder variables between sedentary and active groups for males, we focused on female participants (n=56) to identify differences in the post-vaccination immunology of active and sedentary participants in our subsequent analyse.

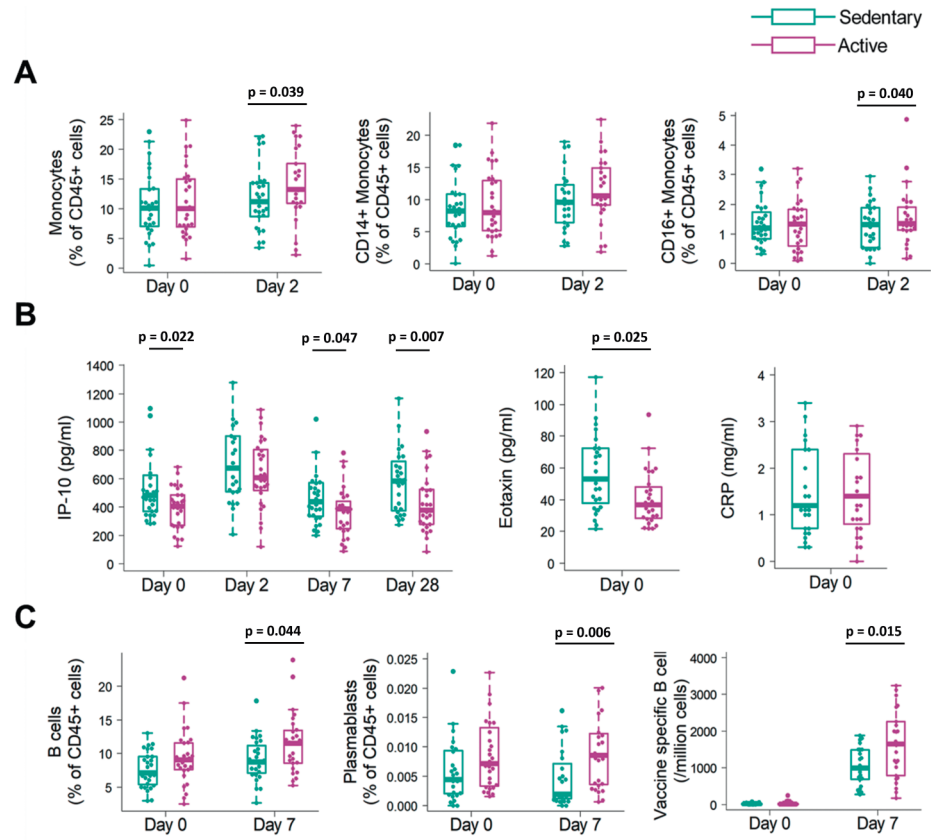


Figure 2. Pre- and Post-Vaccination Immunological Responses in active and sedentary elderly females. (A) Percentages of total monocytes, CD14+ and CD16+ monocytes in active (n=28) and sedentary females (n=28) at D0 and D2. (B) Levels of plasma IP-10 (D0, D2, D7 and D28), Eotaxin (D0) and CRP (D0) in active and sedentary females. (C) Pre- and D7 post-vaccination percentages of B-cells and plasmablasts, as well as the number of vaccine-specific B-cells in active and sedentary females. Percentages of immune cell subsets measured by flow cytometry were compared between sedentary and active individuals using an ANCOVA (analysis of covariance) test with age listed as a covariate. Comparisons across multiple time points within the same set of individuals were performed using the paired Mann-Whitney-Wilcoxon U test. P-values are only indicated where there is a statistically significant difference.

3.2 Kinetics of immune cells and pro-inflammatory cytokines

In active and sedentary females (n=56), we specifically compared the percentages of inflammatory monocytes, classical and non-classical monocytes, eosinophils, neutrophils, dendritic cell subsets and natural killer (NK) cells to identify differences in innate immunity between both populations. Here, the quantity of immune cell subsets as measured by flow cytometry were compared between sedentary and active individuals using an ANCOVA (analysis of covariance) test with age listed as a covariate. The most salient difference was a greater post-vaccination induction of total monocytes (p = 0.039) between active and sedentary elderly females on Day 2 (Figure 2A); this was more statistically significant for non-classical CD16+CD14^{lo} monocytes (ncMono; p = 0.040), although the representation of CD14+CD16- classical monocytes (cMono) was also substantially increased (p = 0.064). We did not observe any changes in the distribution of CD16+CD14+ inflammatory monocytes between active and sedentary elderly women (not shown). In the measurement of pro-inflammatory molecules in

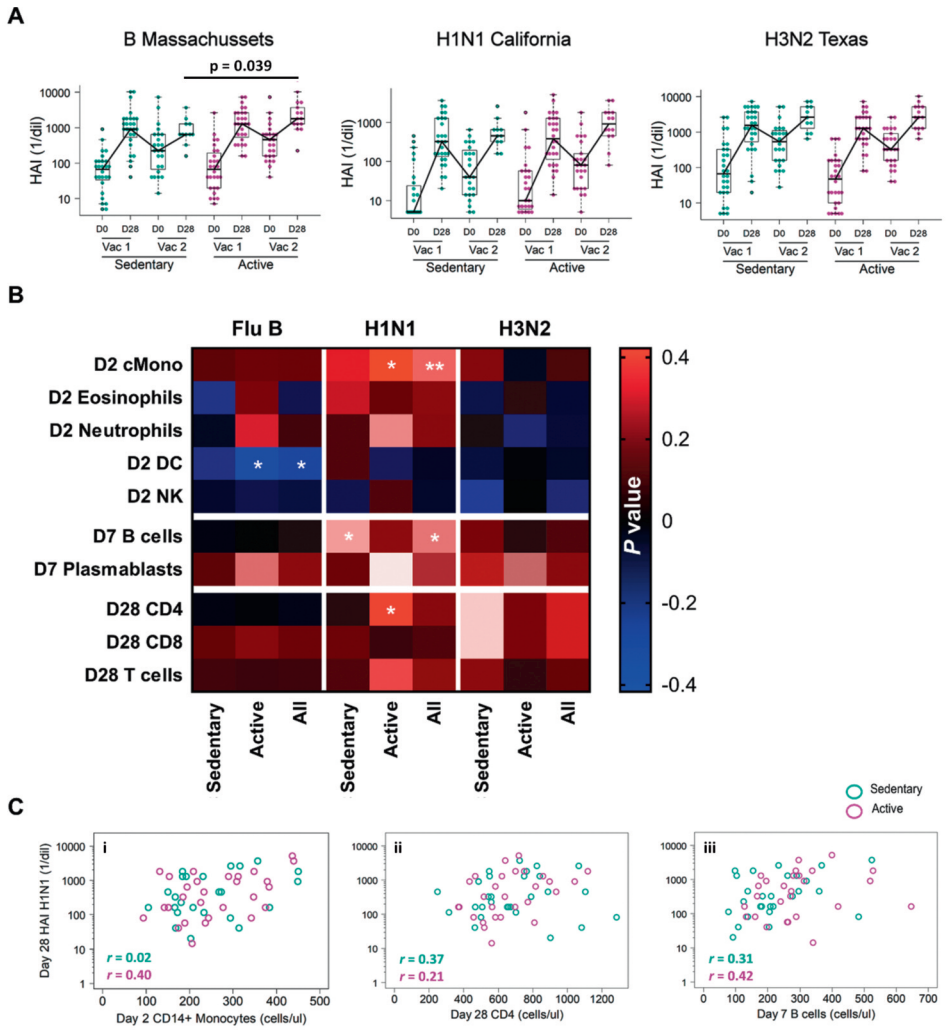


Figure 3. HAI response in active and sedentary elderly females during influenza vaccination. (A) Comparison of strain specific HAI titres between sedentary (green) and active (red) elderly females during the primary (Vac 1) and secondary (Vac 2) vaccination from Baseline (D0) and Day 28 post-vaccination sera (D28). Results for Flu B Massachusetts, H3N1 California and H3N2 Texas are shown. (B) Heatmap corresponding to P-values in the tested correlation between post-vaccination immune cell numbers and D28 HAI titres for all three strains after the first vaccination. P-values of correlation were computed using Pearson's product moment correlation coefficient test in R statistical language using the function cor.test (*P<0.05; **P<0.01). (C) Correlation plots and coefficients for (i) D2 CD14+ monocyte, (ii) D28 CD4 T-cell and (iii) D7 B-cell cell counts versus D28 H1N1 HAI titres. HAI titres and blood counts of immune cells were log2 transformed. Pearson's correlation coefficient between these measurements was calculated across both sedentary and active subject and displayed individually for sedentary (green) and active (red) elderly females.

plasma, there was no statistically significant difference in the levels of high sensitivity C-reactive protein (hsCRP) between sedentary and active elderly females. However, lower levels of the monocyte-derived chemokine, IP-10 (p = 0.022), and eotaxin (p= 0.025) were detected in active elderly females at baseline (Figure 2B).

For adaptive immune cells, we analysed baseline distributions of CD4 and CD8 T-cells and found no statistically significant differences in the numbers of CD4 and CD8 T-cells as well as CD4:CD8 ratios in active and sedentary groups (Table 1). For B-cells, the difference in pre-vaccination percentages of B-cells and plasmablasts (Figure 2C) was not statistically significant between active and sedentary elderly females. However, the Day 7 post-vaccination expansion of B-cells (p = 0.044) and plasmablasts (p = 0.006) was significantly greater in active than in sedentary elderly females. As determined by ELISpot, we also observed an increase in vaccine-specific IgG-secreting B-cells (p = 0.015) within PBMCs that were collected from active females on Day 7 post-vaccination (Figure 2C).

3.3 Post Vaccination HAI Titres

Elderly participants were vaccinated twice in this study, with the second vaccination set at 20 months after receiving the first vaccination. As Vaxigrip® is a trivalent vaccine comprising split virions from three different influenza strains, we investigated HAI titres for each of these strains. Although we did not observe any differences in Day 28 (D28) HAI antibody titres between active and sedentary participants for all three strains in the first vaccination, higher D28 Flu B antibody titres were observed in active females after the second vaccination (Figure 3A; leftmost panel). Subsequently, we assessed whether the post-vaccination numbers of innate and adaptive immune subsets correlated with post-vaccination HAI titres using Pearson's Correlation. Although all three strains were studied (presented as a heat map in Figure 3B), positive correlations between the numbers of immune cell subsets and D28 HAI were only observed for the H1N1 strain after the first vaccination (middle panel; Figure 3B). For active elderly females, the numbers of classical CD14+ monocytes ($p < 0.05$) and CD4 T-cells ($P < 0.05$) at Day 2 (D2) and D28 post-vaccination, respectively, were specifically correlated with D28 HAI titres against the H1N1 strain. The correlation between these immune subsets and H1N1 HAI titres after the first vaccination are resolved in greater detail in Figure 3C, where individual datapoints and r values are individually shown for sedentary (green) and active (red) elderly females (Figures 3C (i) for D2 monocytes and 3C (ii) for D28 CD4 T-cells).

3.4 Transcriptomic Analysis

Finally, we conducted transcriptomic analysis on PBMCs that were collected at baseline to identify differences in gene expression between active and sedentary elderly females. In our microarray analysis, we looked for genes that were differentially regulated between active and sedentary elderly females (Figure 4A). Using a nominal cut off value of $P < 0.05$, we identified 505 DEGs that were further studied using

the Ingenuity Pathway Analysis (34). Here, genetic pathways related to phagocytosis in macrophages/monocytes were highly upregulated in active elderly females relative to their sedentary counterparts (Figure 4B).

4. DISCUSSION

4.1 Summary of Findings and Study Limitations

Opinions on the impact of acute physical activity on the immune system are diverse although many have inferred a state of temporal immunosuppression after acute exercise (76). In addition to lower levels of circulating lymphocytes and antibodies following acute vigorous physical activity, studies have also shown that acute vigorous exercise can increase the risk of infection (8, 53, 54, 60, 76). Although acute physical activity diminishes the frequency of peripheral immune cells, it contributes to the mobilisation of senescent or late differentiated lymphocytes into the periphery (7, 71, 72). Conversely, there is a consensus that frequent physical activity and exercise drives immune competency. Investigations into the relationship between physical activity and improved immunity typically fall into two categories – cross-sectional studies that stratify participants by physical activity level/cardiorespiratory fitness or longitudinal studies that compare immune function after a regular period of physical-activity based interventions. Altogether, these studies describe better responsiveness to mitogen-induced T-cell proliferation, reduced frequencies of senescent cells, higher serum antibody titres following vaccination and cytotoxic activity of NK-cells and neutrophil/monocyte phagocytic function (22, 28, 55, 57, 60, 61).

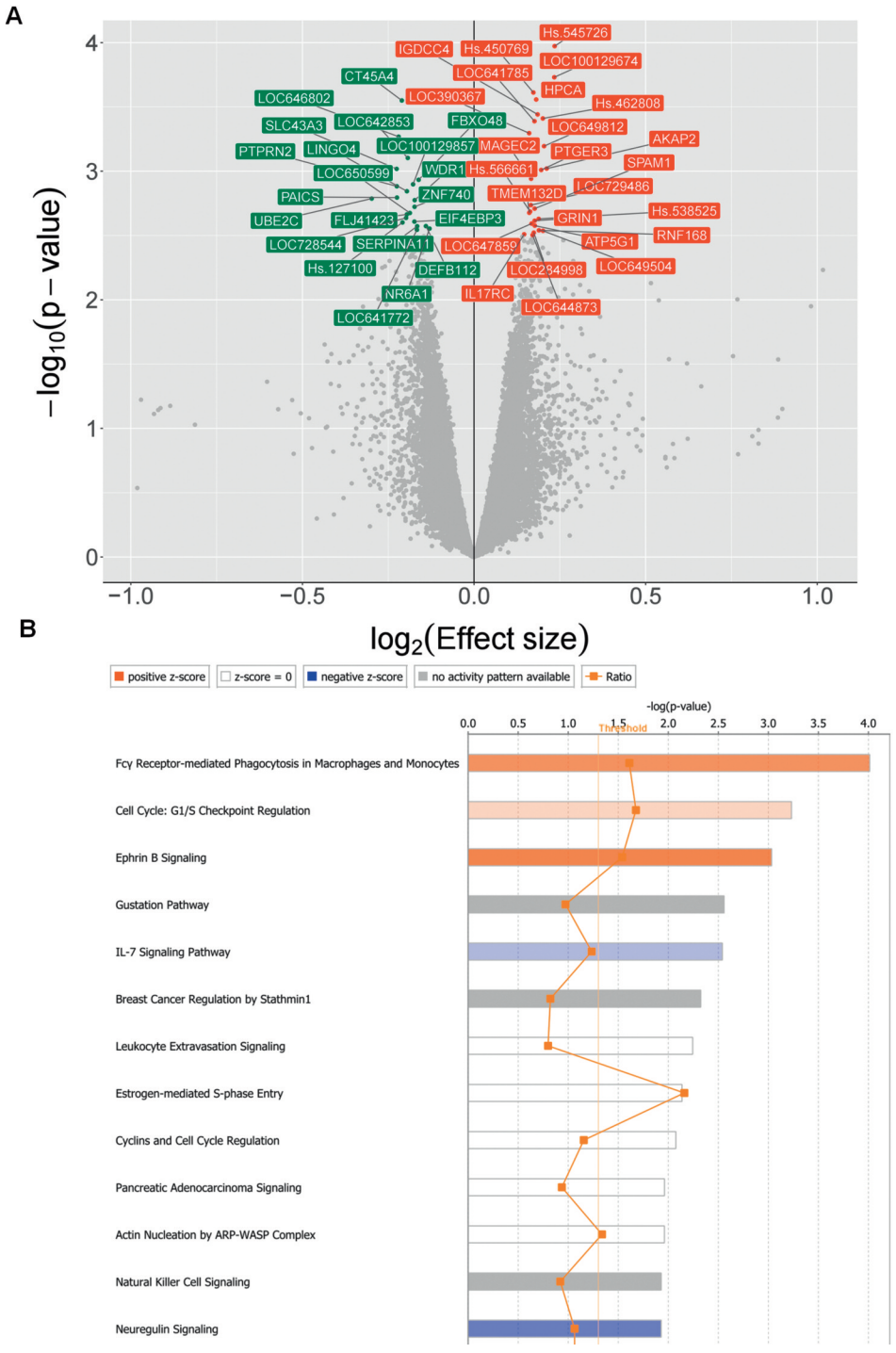


Figure 4. IPA analysis revealing DEGs at baseline in active and sedentary elderly females. (A) 505 DEGs were observed between active and sedentary elderly females using a nominal p value of 0.05 as the cut-off point. (B) Ingenuity Pathway Analysis revealed that genes involved in the regulation of phagocytosis in monocytes and macrophage were most differentially regulated between active and sedentary elderly female participants.

Overall, our findings are consistent with previous literature in describing an augmentation of the post-vaccination immune response in individuals who sustain higher levels of physical activity over extended periods of time. In physically active elderly females, we demonstrate improvements in both arms of the immune response. In addition to a greater post-vaccination expansion of monocytes, we further observed the upregulation of genes related to phagocytic function of monocytes and macrophages, altogether suggesting an enhanced post-vaccination monocyte presence in active elderly females. Furthermore, we describe a stronger post-vaccination plasmablast and vaccine-specific B-cell response in active elderly females as compared to their sedentary counterparts. In active elderly females, the magnitude of the post-vaccination monocyte (D2) and CD4 T-cell (D28) was also found to correlate positively to H1N1 HAI titres, suggesting that the robustness of both innate and adaptive responses is related to the potency of the flu-specific antibody response.

A limitation of this study is that physical activity was only monitored for 14 days after vaccination, however, the design of this study was planned to ensure minimal deviation from their usual routine so that behaviour in these 14 days could be indicative of a prolonged lifestyle in terms of physical activity. Moreover, the generally higher BMC and BMD indices in active females – albeit not statistically significant – suggest long-term differences in physical activity levels between these groups. Thus, it is reasonable to assume that the number of steps completed by each participant are representative of behaviour beyond this 14-day window period. Nevertheless, our study design cannot discriminate whether behavioural history or the amount of physical activity completed within the vaccination window, is responsible for the observed differences in immune activity between active and sedentary elderly women. Since our conclusions are extensively derived from the post-vaccination observation of immune parameters in elderly females, a further limitation is that the improved post-vaccination outcomes influenza vaccination may not be generalisable to males or younger participants.

4.2 Physical Activity and the Immune Response to Influenza Vaccinations

Specific to influenza vaccination, Kohut *et. al.* showed that tri-weekly attendance at a supervised aerobics class contributed to higher antibody titres against H1N1 and H3N2 strains in the elderly (37). While not affecting peak HAI titres, Woods *et. al.* demonstrated that a 10-month cardiovascular exercise-based intervention also benefited the elderly in prolonging the influenza seroprotection period (78). These findings translate poorly to a single bout of cardiovascular exercise, as participation in a brisk walk or a single set of resistance exercise immediately prior to influenza vaccination did not act as an adjuvant to influenza vaccination (20, 44). Although we did not observe any differences in post-vaccination HAI titres between active and sedentary elderly females; the peripheral expansion of monocytes, B-cells, plasmablasts, and vaccine-specific B-cells was more significant in participants who were more active in the immediate post-vaccination period. Curiously, this robust post-vaccination mobilisation of immune subsets was most closely associated with the H1N1 HAI response. A similar strain-specific response has been observed in an earlier trivalent vaccine study, which noted that elderly females mounted stronger antibody responses towards H1N1 when acute exercise was implemented as an adjuvant prior to vaccination (64). In our study, the increased levels of physical activity could contribute to detectable benefits in the immune response against H1N1 as participants had the lowest baseline HAI and seroprotection rates against H1N1.

The lack of convergence with earlier studies, with respect to improvements in post-vaccination HAI titres, could likewise be a result of the high levels of pre-vaccination seroprotection rates and HAI titres against Flu B and H3N2 (>50%) across both active and sedentary groups (36). Since most elderly subjects in our study responded robustly to influenza vaccination and seroconverted, it was less likely for differences in HAI titres between active and sedentary females to be detected. An alternative explanation may relate to the intensity or regularity of physical activity required to boost post-vaccination antibody titres, as participants were not subjected to any organised or specific intervention programmes. Unlike earlier studies that grouped donors based on intervention arms or physical activity levels prior to vaccination, donor stratification was based on post-vaccination physical activity levels in this study (15, 36, 37, 69, 78). Nevertheless, we introduce many important perspectives to the link between physical activity and post-vaccination immunity. We demonstrate firstly that the augmentation of post-vaccination HAI titres by physical activity may be closely related to changes in innate immunity – or more specifically monocyte trafficking and phagocytosis. Consistent with the upregulation of genes associated with macrophage and monocyte phagocytosis in PBMCs from active elderly females, many studies have revealed that physical activity enhances the phagocytic potential of these innate immune cells (22, 57). Since phagocytosis is fundamental to antigen-processing and presentation, improvements in the former are likely to directly benefit the generation of antigen-specific T- and B-cell immune responses (22). Serving as a potential cellular reservoir for subsequent differentiation towards specialised antigen-presenting macrophages and dendritic cells, the greater post-vaccination expansion of the monocyte pool is also likely to have contributed to the increased post-vaccination frequencies of plasmablasts and vaccine-specific B-cells in the active elderly female group.

As participants were administered two rounds of Vaxigrip© vaccination in our study – the second after a 20-month interval – we were able to assess the influence of physical activity on repeated immunisations. That the physically active elderly female group could mount a superior antibody response to Flu B after the second vaccination suggests that they possess better preservation and persistence of memory B-cell plasticity and function. The higher pre-vaccination proportions of B-cells and plasmablasts – and generation of vaccine specific B-cells – during the first vaccination are consistent with this hypothesis and may further reflect improvements in hematopoietic potential in active elderly females. Although not statistically significant, we observed higher HAI titres against Flu B and H1N1 immediately prior to the second vaccination, suggesting that active elderly females may accommodate better antibody persistence in the 20-month post-vaccination period as compared to sedentary participants. The present literature is supportive of the above interpretations. Although the mechanisms are unclear, regular exercise promotes haematopoiesis and contributes to higher numbers of hematopoietic stem cells in the bone marrow and peripheral blood (3, 5). Moreover, cyclists have been reported to possess higher levels of the hematopoietic cytokine, IL-7, and frequencies of recent thymic emigrants in their blood (19). Specific to the B-cell response, de Araújo *et. al.* had also observed more potent and durable antibody responses to influenza vaccination in elderly men with a moderate or intense training lifestyle (15). Finally, reports have highlighted that those who exercise have higher circulating antibody titres (IgM and class-switched isotypes) suggesting that regular physical activity is important for memory B-cell/plasmablast function (49, 56).

4.3 Physical Activity in the Alleviation of 'Inflammaging'

Aging is associated with the persistence of low-grade chronic inflammation – otherwise known as "inflammaging"; this is

characterised by increased levels of pro-inflammatory molecules (IL-6, CRP, IL-1β, IL-15 and TNF-α) and levels of systemic inflammation have shown close association with the development of comorbidities in the elderly – including cardiovascular diseases, type-2 diabetes, Alzheimer's disease, osteoporosis and multiple cancer types (23, 24, 27). Moreover, elevated systemic levels of IL-6 and CRP have been shown to predict mortality in longitudinal studies that include nonagenarians (4, 26, 34). Since immune activation is a key driver of immunosenescence and loss of lymphocyte function, we may well speculate that the lower levels of systemic inflammation (characterised by IP-10 and eotaxin levels) have a critical role to play in driving the higher immune responses observed in active elderly females from our influenza vaccine study. Other studies have highlighted the negative relationships between regular physical activity and systemic IL-6, CRP and TNF-α levels in the elderly (29, 34). Although we did not observe any differences in CRP levels between sedentary and active elderly females, lower levels of pro-inflammatory IFN-γ-induced protein precursors (IP-10 or CXCL-10) and eotaxin were detected in more active females. IP-10 is a potent monocyte- or dendritic cell-derived chemoattractant that recruits activated T-cells to secondary lymphoid tissues and inflammatory sites of action (18). While a decrease in eotaxin levels following a 12-week exercise intervention period has been illustrated with a cohort of Korean women, the relationship between physical activity and IP-10 levels has not been previously described (12).

Since IP-10 is a pro-inflammatory marker that is associated with susceptibility towards infectious diseases, autoimmunity and cancer – lower baseline levels of IP-10 may protect against these pathologies (1, 42, 43). In our study, we further observed that active elderly females achieved better post-vaccination resolution of IP-10 levels – noticeable as early as 7 days post-vaccination in a trend that persists even at 28 days post-vaccination. As we monitored walking activity in the period immediately following vaccination, the swift resolution of IP-10 could be directly related to the levels of physical activity attained by elderly female participants during the period of vaccination. It is important to note that the lower baseline levels of IP-10 in active elderly females do not imply an impaired capacity to secrete IP-10, as active elderly females equally upregulated plasma IP-10 levels on Day 2 post-vaccination. The induction of interferon-induced genes, particularly IP-10 in the first days of vaccination, has been shown to be a critical component of the predictive signature of influenza, yellow fever and Ebola vaccine efficacy (2, 16, 47, 63, 66). In an investigation of the influenza vaccination response, Athale and colleagues also found that vaccine efficacy was highly related to the rapid in vitro secretion of type 1 IFN and IP-10 by innate immune cells (2).

4.4 Improving Health in the Elderly through Physical Activity

While physical activity may have a significant role in lowering the risk of many age-related diseases (10, 75), we did not observe many differences in the frequency of co-morbidities between active and sedentary elderly individuals in our community dwelling cohort. For example, in addition to diabetic and arthritic status, blood pressure and cholesterol levels were not statistically different between active and sedentary elderly individuals in this study. Nevertheless, we observed that sarcopenic status greatly differed between active and sedentary elderly males. While a multitude of cross-sectional and cohort studies have shown that regular physical activity reduces the risk of developing sarcopenia, the cross-sectional nature of our study makes it difficult to determine the directional relationship between sarcopenic status and levels of physical activity among our male participants. Recent evidence, however, indicates that by delaying the decay of skeletal muscle mass and function and improving muscular strength and power, physical activity and exercise are effective intervention strategies that prevent the development of sarcopenia (32, 74). A comparison of DEXA measurements between sedentary and active elderly individuals in our study reveals trends that support this relationship between physical activity and the development of sarcopenia. More specifically, indices related to muscular and skeletal health were strengthened in active elderly participants relative to their sedentary counterparts.

In terms of monitoring the impact of physical activity on vaccination outcomes, our study is the first to describe improved cell-mediated responses using an accelerometer-based study that records physical activity levels in the immediate post-vaccination period. While there is a strong case for promoting physical activity as a fundamental component to healthy aging, the results of previous studies display a heavy reliance on self-reported physical activity or interventions featuring organised exercise regimes. The complexity and type of exercise training (cardiovascular, aerobic training, resistance training, power training) also contribute to contrasting observations, and it is therefore challenging to interpret the value of organised interventions or implement them on a national level (30, 60). The rapidly growing proportion of elderly individuals (aged 65 years or older) worldwide, however, create a pressing need for institutionalised interventions that delay the acquisition of age-related co-morbidities. For the elderly, the WHO has suggested that a weekly recommendation of at least 150 minutes of moderate-intensity aerobic activity or 75 minutes of vigorous-intensity aerobic activity is necessary to achieve the health benefits associated with physical activity (81). Here, the use of pedometers and accelerometers provide practical and reliable methods for tracking and incentivising physical activity among the elderly.

As part of a national initiative to promote physical activity, the Singapore Health Promotion Board has organised a National Steps Challenge, which awards modest financial rewards to motivate participants to become more physically active and has seen 690,000 sign-ups in 2018 alone. Although its impact on national health has not been studied, the high participation rate suggests the feasibility of implementing a pedometer-based programme to boost national health. It could be critical for other countries to implement similar policies to reverse the high prevalence rates of insufficient physical activity (80, 81). Overall, our demonstration of the benefits of physical activity on immune responses to influenza vaccination in elderly women suggest that exercise can be a safe and valuable adjunct to interventions that aim to promote immune robustness in older individuals.

Author Contributions

AL, NBu, BA, TPN and PT conceptualised the design of this vaccine study. GW, Nbo and AL planned the analysis for the exercise component of this study. GW, XC, CT and AL designed and performed the immunological assays. GW analysed the data and wrote the manuscript. VN performed the statistical analyses described in this study. All other authors participated in the collection, analysis and interpretation of data. All authors approved the final version of this manuscript.

Disclosure of Funding

The study was supported by research grants from Sanofi-Pasteur, Nestle and the Agency for Science Technology and Research (A*STAR, including the National Medical Research Council [NMRC/1108/2007], Biomedical Research Council (BMRC) [08/1/21/19/567] and Joint Council Office (JCO) [1434m0015) grants. SIgN Immunomonitoring is further supported by BMRC grants and transition funds [IAF 311006 and #H16/99/b0/011 respectively].

Ethics Approval

The National Healthcare Group (NHG) Domain Specific Institutional Review Board (DSRB) approved a phase IV clinical trial of Sanofi Pasteur's Vaxigrip© influenza vaccine, which is registered at clinicaltrials.gov under the registration number NCT03266237. All volunteers provided written informed consent.

Conflict of Interest Statement

The authors declare a potential conflict of interest: Christope Carre and Laurence Quemeneur are employees of Sanofi-Pasteur and Nabil Bosco is an employee of Nestle Research Centre, Singapore. All other authors declare no competing interests.

Acknowledgements

The authors thank all research nurses for their involvement in sample collection and questionnaire administration.

REFERENCES

1. Antonelli A, Rotondi M, Fallahi P, Romagnani P, Ferrari SM, Buonamano A, Ferrannini E, and Serio M. High levels of circulating CXC chemokine ligand 10 are associated with chronic autoimmune thyroiditis and hypothyroidism. J Clin Endocrinol Metab 89: 5496-5499, 2004.
2. Athale S, Banchereau R, Thompson-Snipes L, Wang Y, Palucka K, Pascual V, and Banchereau J. Influenza vaccines differentially regulate the interferon response in human dendritic cell subsets. Sci Transl Med 9: 2017.
3. Baker JM, De Lisio M, and Parise G. Endurance exercise training promotes medullary hematopoiesis. FASEB J 25: 4348-4357, 2011.
4. Bologa RM, Levine DM, Parker TS, Cheigh JS, Serur D, Stenzel KH, and Rubin AL. Interleukin-6 predicts hypoalbuminemia, hypocholesterolemia, and mortality in hemodialysis patients. Am J Kidney Dis 32: 107-114, 1998.
5. Bonsignore MR, Morici G, Santoro A, Pagano M, Cascio L, Bonanno A, Abate P, Mirabella F, Profita M, Insalaco G, Gioia M, Vignola AM, Majolino I, Testa U, and Hogg JC. Circulating hematopoietic progenitor cells in runners. J Appl Physiol (1985) 93: 1691-1697, 2002.
6. Bross R, Javanbakht M, and Bhasin S. Anabolic interventions for aging-associated sarcopenia. J Clin Endocrinol Metab 84: 3420-3430, 1999.
7. Campbell JP, Riddell NE, Burns VE, Turner M, van Zanten JJ, Drayson MT, and Bosch JA. Acute exercise mobilises CD8+ T lymphocytes exhibiting an effector-memory phenotype. Brain Behav Immun 23: 767-775, 2009.
8. Campbell JP, and Turner JE. Debunking the Myth of Exercise-Induced Immune Suppression: Redefining the Impact of Exercise on Immunological Health Across the Lifespan. Front Immunol 9: 648, 2018.
9. Caspersen CJ. Physical activity epidemiology: concepts, methods, and applications to exercise science. Exerc Sport Sci Rev 17: 423-473, 1989.
10. Caspersen CJ, Powell KE, and Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep 100: 126-131, 1985.
11. Chen LK, Liu LK, Woo J, Assantachai P, Auyeung TW, Bahyah KS, Chou MY, Chen LY, Hsu PS, Krairit O, Lee JS, Lee WJ, Lee Y, Liang CK, Limpawattana P, Lin CS, Peng LN, Satake S, Suzuki T, Won CW, Wu CH, Wu SN, Zhang T, Zeng P, Akishita M, and Arai H. Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. J Am Med Dir Assoc 15: 95-101, 2014.
12. Choi KM, Kim JH, Cho GJ, Baik SH, Park HS, and Kim SM. Effect of exercise training on plasma visfatin and eotaxin levels. Eur J Endocrinol 157: 437-442, 2007.
13. Clough E, and Barrett T. The Gene Expression Omnibus Database. Methods Mol Biol 1418: 93-110, 2016.
14. Daley MJ, and Spinks WL. Exercise, mobility and aging. Sports Med 29: 1-12, 2000.
15. de Araujo AL, Silva LC, Fernandes JR, Matias Mde S, Boas LS, Machado CM, Garcez-Leme LE, and Benard G. Elderly men with moderate and intense training lifestyle present sustained higher antibody responses to influenza vaccine. Age (Dordr) 37: 105, 2015.
16. Diaz-Mitoma F, Alvarez-Maya I, Dabrowski A, Jaffey J, Frost R, Aucoin S, Kryworuchko M, Lapner M, Tadesse H, and Giulivi A. Transcriptional analysis of human peripheral blood mononuclear cells after influenza immunization. J Clin Virol 31: 100-112, 2004.
17. Du P, Kibbe WA, and Lin SM. lumi: a pipeline for processing Illumina microarray. Bioinformatics 24: 1547-1548, 2008.
18. Dufour JH, Dziejman M, Liu MT, Leung JH, Lane TE, and Luster AD. IFN-gamma-inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. J Immunol 168: 3195-3204, 2002.
19. Duggal NA, Pollock RD, Lazarus NR, Harridge S, and Lord JM. Major features of immunesenescence, including reduced thymic output, are ameliorated by high levels of physical activity in adulthood. Aging Cell 17: 2018.
20. Edwards KM, Pascoe AR, Fiatarone-Singh MA, Singh NA, Kok J, and Booy R. A randomised controlled trial of resistance exercise prior to administration of influenza vaccination in older adults. Brain Behav Immun 49: e24-e25, 2015.
21. Eijssen LM, Goelela VS, Kelder T, Adriaens ME, Evelo CT, and Radonjic M. A user-friendly workflow for analysis of Illumina gene expression bead array data available at the arrayanalysis.org portal. BMC Genomics 16: 482, 2015.

22. Fehr HG, Lotzerich H, and Michna H. The influence of physical exercise on peritoneal macrophage functions: histochemical and phagocytic studies. Int J Sports Med 9: 77-81, 1988.
23. Franceschi C, and Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci 69 Suppl 1: S4-9, 2014.
24. Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, Panourgia MP, Invidia L, Celani L, Scurti M, Cevenini E, Castellani GC, and Salvioli S. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech Ageing Dev 128: 92-105, 2007.
25. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, McBurnie MA, and Cardiovascular Health Study Collaborative Research G. Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 56: M146-156, 2001.
26. Frink M, van Griensven M, Kobbe P, Brin T, Zeckey C, Vaske B, Krettek C, and Hildebrand F. IL-6 predicts organ dysfunction and mortality in patients with multiple injuries. Scand J Trauma Resusc Emerg Med 17: 49, 2009.
27. Giunta B, Fernandez F, Nikolic WV, Obregon D, Rrapo E, Town T, and Tan J. Inflammaging as a prodrome to Alzheimer's disease. J Neuroinflammation 5: 51, 2008.
28. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, and Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nat Rev Immunol 11: 607-615, 2011.
29. Gleeson M, Pyne DB, McDonald WA, Clancy RL, Cripps AW, Horn PL, and Fricker PA. Pneumococcal antibody responses in elite swimmers. Clin Exp Immunol 105: 238-244, 1996.
30. Goodwin K, Viboud C, and Simonsen L. Antibody response to influenza vaccination in the elderly: a quantitative review. Vaccine 24: 1159-1169, 2006.
31. Heymsfield SB, Smith R, Aulet M, Bensen B, Lichtman S, Wang J, and Pierson RN, Jr. Appendicular skeletal muscle mass: measurement by dual-photon absorptiometry. Am J Clin Nutr 52: 214-218, 1990.
32. Iolascon G, Di Pietro G, Gimigliano F, Mauro GL, Moretti A, Giamattei MT, Ortolani S, Tarantino U, and Brandi ML. Physical exercise and sarcopenia in older people: position paper of the Italian Society of Orthopaedics and Medicine (OrtoMed). Clin Cases Miner Bone Metab 11: 215-221, 2014.
33. Jeon CY, Lokken RP, Hu FB, and van Dam RM. Physical activity of moderate intensity and risk of type 2 diabetes: a systematic review. Diabetes Care 30: 744-752, 2007.
34. Jylha M, Paavilainen P, Lehtimaki T, Goebeler S, Karhunen PJ, Hervonen A, and Hurme M. Interleukin-1 receptor antagonist, interleukin-6, and C-reactive protein as predictors of mortality in nonagenarians: the vitality 90+ study. J Gerontol A Biol Sci Med Sci 62: 1016-1021, 2007.
35. Keylock KT, Lowder T, Leifheit KA, Cook M, Mariani RA, Ross K, Kim K, Chapman-Novakofski K, McAuley E, and Woods JA. Higher antibody, but not cell-mediated, responses to vaccination in high physically fit elderly. J Appl Physiol (1985) 102: 1090-1098, 2007.
36. Kohut ML, Arntson BA, Lee W, Rozeboom K, Yoon KJ, Cunnick JE, and McElhaney J. Moderate exercise improves antibody response to influenza immunization in older adults. Vaccine 22: 2298-2306, 2004.
37. Kohut ML, Cooper MM, Nickolaus MS, Russell DR, and Cunnick JE. Exercise and psychosocial factors modulate immunity to influenza vaccine in elderly individuals. J Gerontol A Biol Sci Med Sci 57: M557-562, 2002.
38. Kramer A, Green J, Pollard J, Jr., and Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. Bioinformatics 30: 523-530, 2014.
39. Larbi A, and Fulop T. From "truly naive" to "exhausted senescent" T cells: when markers predict functionality. Cytometry A 85: 25-35, 2014.
40. Lawton MP, and Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist 9: 179-186, 1969.
41. Lewis RJ, and Endean R. Direct and indirect effects of ciguatoxin on guinea-pig atria and papillary muscles. Naunyn Schmiedebergs Arch Pharmacol 334: 313-322, 1986.
42. Liu M, Guo S, Hibbert JM, Jain V, Singh N, Wilson NO, and Stiles JK. CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. Cytokine Growth Factor Rev 22: 121-130, 2011.
43. Liu M, Guo S, and Stiles JK. The emerging role of CXCL10 in cancer (Review). Oncol Lett 2: 583-589, 2011.
44. Long JE, Ring C, Drayson M, Bosch J, Campbell JP, Bhabra J, Browne D, Dawson J, Harding S, Lau J, and Burns VE. Vaccination response following aerobic exercise: can a brisk walk enhance antibody response to pneumococcal and influenza vaccinations? Brain Behav Immun 26: 680-687, 2012.
45. Lord SR, Menz HB, and Tiedemann A. A physiological profile approach to falls risk assessment and prevention. Phys Ther 83: 237-252, 2003.
46. Minuzzi LG, Rama L, Chupel MU, Rosado F, Dos Santos JV, Simpson R, Martinho A, Paiva A, and Teixeira AM. Effects of lifelong training on senescence and mobilization of T lymphocytes in response to acute exercise. Exerc Immunol Rev 24: 72-84, 2018.
47. Nakaya HI, Wrammert J, Lee EK, Racioppi L, Marie-Kunze S, Haining WN, Means AR, Kasturi SP, Khan N, Li GM, McCausland M, Kanchan V, Kokko KE, Li S, Elbein R, Mehta AK, Aderem A, Subbarao K, Ahmed R, and Pulendran B. Systems biology of vaccination for seasonal influenza in humans. Nat Immunol 12: 786-795, 2011.
48. Nasreddine ZS, Phillips NA, Bedirian V, Charbonneau S, Whitehead V, Collin I, Cummings JL, and Chertkow H. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 53: 695-699, 2005.
49. Nehlsen-Cannarella SL, Nieman DC, Balk-Lamberton AJ, Markoff PA, Chritton DB, Gusewitch G, and Lee JW. The effects of moderate exercise training on immune response. Med Sci Sports Exerc 23: 64-70, 1991.
50. Nelson HD, Nevitt MC, Scott JC, Stone KL, and Cummings SR. Smoking, alcohol, and neuromuscular and physical function of older women. Study of Osteoporotic Fractures Research Group. JAMA 272: 1825-1831, 1994.
51. Ng TP, Feng L, Nyunt MS, Larbi A, and Yap KB. Frailty in older persons: multisystem risk factors and the Frailty Risk Index (FRI). J Am Med Dir Assoc 15: 635-642, 2014.
52. Nicklas BJ, Hsu FC, Brinkley TJ, Church T, Goodpaster BH, Kritchevsky SB, and Pahor M. Exercise training and plasma C-reactive protein and interleukin-6 in elderly people. J Am Geriatr Soc 56: 2045-2052, 2008.

53. Nieman DC. Exercise and resistance to infection. Can J Physiol Pharmacol 76: 573-580, 1998.
54. Nieman DC. Exercise, infection, and immunity. Int J Sports Med 15 Suppl 3: S131-141, 1994.
55. Nieman DC. Special feature for the Olympics: effects of exercise on the immune system: exercise effects on systemic immunity. Immunol Cell Biol 78: 496-501, 2000.
56. Nieman DC, and Nehlsen-Cannarella SL. The effects of acute and chronic exercise of immunoglobulins. Sports Med 11: 183-201, 1991.
57. Ortega Rincon E. Physiology and biochemistry: influence of exercise on phagocytosis. Int J Sports Med 15 Suppl 3: S172-178, 1994.
58. Pachana NA, Byrne GJ, Siddle H, Koloski N, Harley E, and Arnold E. Development and validation of the Geriatric Anxiety Inventory. Int Psychogeriatr 19: 103-114, 2007.
59. Pangman VC, Sloan J, and Guse L. An examination of psychometric properties of the mini-mental state examination and the standardized mini-mental state examination: implications for clinical practice. Appl Nurs Res 13: 209-213, 2000.
60. Pascoe AR, Fiatarone Singh MA, and Edwards KM. The effects of exercise on vaccination responses: a review of chronic and acute exercise interventions in humans. Brain Behav Immun 39: 33-41, 2014.
61. Pedersen BK, and Ullum H. NK cell response to physical activity: possible mechanisms of action. Med Sci Sports Exerc 26: 140-146, 1994.
62. Petersen AM, and Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol (1985) 98: 1154-1162, 2005.
63. Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, Pirani A, Gernert K, Deng J, Marzolf B, Kennedy K, Wu H, Bennouna S, Oluoch H, Miller J, Vencio RZ, Mulligan M, Aderem A, Ahmed R, and Pulendran B. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. Nat Immunol 10: 116-125, 2009.
64. Ranadive SM, Cook M, Kappus RM, Yan H, Lane AD, Woods JA, Wilund KR, Iwamoto G, Vanar V, Tandon R, and Fernhall B. Effect of acute aerobic exercise on vaccine efficacy in older adults. Med Sci Sports Exerc 46: 455-461, 2014.
65. Randolph GJ, Jakubzick C, and Qu C. Antigen presentation by monocytes and monocyte-derived cells. Curr Opin Immunol 20: 52-60, 2008.
66. Rechtien A, Richert L, Lorenzo H, Martrus G, Hejblum B, Dahlke C, Kasonta R, Zinser M, Stubbe H, Matschl U, Lohse A, Krahling V, Eickmann M, Becker S, Consortium V, Thiebaut R, Altfeld M, and Addo MM. Systems Vaccinology Identifies an Early Innate Immune Signature as a Correlate of Antibody Responses to the Ebola Vaccine rVSV-ZEBOV. Cell Rep 20: 2251-2261, 2017.
67. Ringseis R, Eder K, Mooren FC, and Kruger K. Metabolic signals and innate immune activation in obesity and exercise. Exerc Immunol Rev 21: 58-68, 2015.
68. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, and Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43: e47, 2015.
69. Schuler PB, Leblanc PA, and Marzilli TS. Effect of physical activity on the production of specific antibody in response to the 1998-99 influenza virus vaccine in older adults. J Sports Med Phys Fitness 43: 404, 2003.
70. Schuler PB, Lloyd LK, Leblanc PA, Clapp TA, Abadie BR, and Collins RK. The effect of physical activity and fitness on specific antibody production in college students. J Sports Med Phys Fitness 39: 233-239, 1999.
71. Simpson RJ, Cosgrove C, Chee MM, McFarlin BK, Bartlett DB, Spielmann G, O'Connor DP, Pircher H, and Shiels PG. Senescent phenotypes and telomere lengths of peripheral blood T-cells mobilized by acute exercise in humans. Exerc Immunol Rev 16: 40-55, 2010.
72. Simpson RJ, Florida-James GD, Cosgrove C, Whyte GP, Macrae S, Pircher H, and Guy K. High-intensity exercise elicits the mobilization of senescent T lymphocytes into the peripheral blood compartment in human subjects. J Appl Physiol (1985) 103: 396-401, 2007.
73. Spielmann G, McFarlin BK, O'Connor DP, Smith PJ, Pircher H, and Simpson RJ. Aerobic fitness is associated with lower proportions of senescent blood T-cells in man. Brain Behav Immun 25: 1521-1529, 2011.
74. Steffl M, Bohannon RW, Sontakova L, Tufano JJ, Shiells K, and Holmerova I. Relationship between sarcopenia and physical activity in older people: a systematic review and meta-analysis. Clin Interv Aging 12: 835-845, 2017.
75. Taylor D. Physical activity is medicine for older adults. Postgrad Med J 90: 26-32, 2014.
76. Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop NC, Fleshner M, Green C, Pedersen BK, Hoffman-Goetz L, Rogers CJ, Northoff H, Abbasi A, and Simon P. Position statement. Part one: Immune function and exercise. Exerc Immunol Rev 17: 6-63, 2011.
77. Ware J, Jr., Kosinski M, and Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. Med Care 34: 220-233, 1996.
78. Woods JA, Keylock KT, Lowder T, Vieira VJ, Zelkovich W, Dumich S, Colantuano K, Lyons K, Leifheit K, Cook M, Chapman-Novakofski K, and McAuley E. Cardiovascular exercise training extends influenza vaccine seroprotection in sedentary older adults: the immune function intervention trial. J Am Geriatr Soc 57: 2183-2191, 2009.
79. World Health Organisation. Global Health and Aging, 2011. http://www.who.int/ageing/publications/global_health.pdf. [September 4, 2018].
80. World Health Organisation. Global health risks: mortality and burden of disease attributable to selected major risks, 2009. http://www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_full.pdf. [September 4, 2018].
81. World Health Organisation. Global recommendations on physical activity for health, 2010. http://www.who.int/dietphysicalactivity/publications/9789241599979/en/ [September 4, 2018].
82. Yesavage JA. Geriatric Depression Scale. Psychopharmacol Bull 24: 709-711, 1988.

T and B cell subsets differentially correlate with amyloid deposition and neurocognitive function in patients with amnestic mild cognitive impairment after one year of physical activity

Katherine Poinsatte[1], Emily E Smith[2], Vanessa O Torres[1], Sterling B Ortega[1], PhD, Ryan M Huebinger[3], PhD, C Munro Cullum[1,2], PhD, Nancy L Monson[1,4], PhD, Rong Zhang[1,5], PhD and Ann M Stowe[1,6], PhD

1 Dept. of Neurology & Neurotherapeutics, UT Southwestern Medical Center, 6000 Harry Hines, Dallas, TX, 75390, USA
2 Dept. of Psychiatry, UT Southwestern Medical Center, 5323 Harry Hines, Dallas, TX, 75390, USA
3 Dept. of Surgery, UT Southwestern Medical Center, 6000 Harry Hines, Dallas, TX, 75390, USA
4 Dept. of Immunology, UT Southwestern Medical Center, 6000 Harry Hines, Dallas, TX, 75390, USA
5 Insitute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital, 7232 Greenville Ave., Dallas, TX, 75231, USA
6 Dept. of Neurology, University of Kentucky, 741 S. Limestone St, Lexington, KY, 40506, USA

ABSTRACT

Individuals with amnestic mild cognitive impairment (aMCI) experience cognitive declines in learning and memory greater than expected for normal aging, and are at a high risk of dementia. We previously reported that sedentary aMCI patients exhibited neuroinflammation that correlated with brain amyloid beta (Aβ) burden, as determined by ^{18}F-florbetapir positron emission tomography (PET). These aMCI patients enrolled in a one-year randomized control trial (AETMCI, NCT01146717) to test the beneficial effects of 12 months of moderate-to-high intensity aerobic exercise training (AET) or stretching/toning (ST) control intervention on neurocognitive function. A subset of aMCI participants had PET imaging, cognitive testing, and immunophenotyping of cerebrospinal fluid (CSF) and peripheral blood after AET or ST interventions. As adaptive immune responses were similar between AET and ST groups, we combined AET/ST into a general 'physical activity' (PA) group and compared Aβ burden, cognitive function, and adaptive immune cell subsets to sedentary lifestyle before intervention. We found that PA-induced immunomodulation of CD4$^+$ and CD8$^+$ T cells in CSF correlated with changes in Aβ burden in brain regions associated with executive function. Furthermore, after PA, cognitive scores on tests of memory, processing speed, attention, verbal fluency, and executive function were associated with increased percent representation of circulating naïve B cells and CD8$^+$ T cells. We review the literature on aMCI-related cognition and immune changes as they relate to exercise, and highlight how our preliminary data suggest a complex interplay between the adaptive immune system, physical activity, cognition, and Aβ burden in aMCI.

Key words: amnestic mild cognitive impairment, physical activity, adaptive immunity, lymphocytes, PET amyloid imaging

Corresponding Author:
Dr. Ann M. Stowe, University of Kentucky; ann.stowe@uky.edu

INTRODUCTION

Individuals with amnestic mild cognitive impairment (aMCI) experience cognitive decline greater than expected for normal aging, and exhibit primary impairments in learning and memory (1). More than half of patients with aMCI exhibit increased amyloid beta (Aβ) deposition in the brain and progress to dementia (2, 3). Currently, there are no approved therapies to prevent and protect against aMCI (4), but a growing body of literature provides evidence for the beneficial effects of exercise interventions (5). Physical activity (PA) has been reported to reduce the incidence of aMCI, as well as improve cognitive function in multiple domains (6), including global cognition, attention, executive functioning, verbal fluency, and memory (7-11).

One mechanism that may explain the positive effects of PA in aMCI patients is exercise-induced alterations within the immune system. To date, only 3 studies have investigated whether exercise modulates inflammation in patients with MCI (12-14), primarily focusing on post-exercise serum concentrations of cytokines, small molecules secreted by leukocytes that impact the function and phenotype of other immune cells (12-14). While these studies utilized different exercise training regimens, they generally conclude that exercise increases anti-inflammatory cytokines while concomitantly reducing pro-inflammatory cytokine serum levels in patients with MCI (12-14). The one study that examined changes in leukocytes after exercise found circulating lymphocyte (i.e. adaptive immune cell) populations reduced by 28 weeks of strength training in MCI patients (12). Together, these studies suggest that exercise impacts the immune system of aMCI patients, but provide limited information about the effect of PA on particular immune cell subsets, specifically B and T cells, and how immune changes after PA may be related to cognitive performance.

Previously, we examined baseline data from an aMCI cohort enrolled in a one-year randomized control trial (AETMCI, NCT01146717) to test the efficacy of 12 months of moderate-to-high intensity aerobic exercise training (AET) on cognition compared to a stretching/toning (ST) control group. Partici-

pants underwent AET that progressively increased in intensity for 26 weeks, at which point they underwent 4-5 exercise sessions weekly with 2-3 moderate intensity training sessions (75-85% of maximal heart rate) and 2 high intensity sessions (85-90% of maximal heart rate). ST cohorts received similar attention from, and interactions with, investigators and underwent weekly bouts of stretching and toning (<50% of maximal heart rate). We found that sedentary aMCI patients exhibited elevated immune cells in the CSF (15) that correlated with increased Aβ burden in the brain, as determined by ^{18}F-florbetapir positron emission tomography (PET) (16). The analysis of adaptive immune cells within the AETMCI trial was only available during the final year of enrollment, and thus did not include the entire aMCI patient data set. Nevertheless, we sought to elucidate whether PA altered adaptive immune cell subsets in individuals with aMCI. Specifically, we examined how PA affected B and T cells in the blood and CSF, and whether immunomodulation was associated with changes in either cognitive function or brain Aβ burden. Collectively, our preliminary data suggested a complex interplay between the adaptive immune system, cognition, and Aβ burden in aMCI patients and support the hypothesis that PA could be a beneficial therapeutic intervention for patients with aMCI.

METHODS

Participant characteristics: All subjects enrolled in this study (15-17) provided written informed consent approved by UT Southwestern (UTSW) Medical Center and Texas Health Presbyterian Hospital of Dallas, Texas Institutional Review Boards. Subjects enrolled in the AETMCI (NCT01146717) clinical trial gave additional consent for lumbar puncture, blood draw, and PET imaging. The diagnosis of aMCI was based upon standard Petersen criteria (18), as modified from the Alzheimer's Disease Neuroimaging Initiative (ADNI) project (http://adni-info.org). Diagnostic criteria included a global Clinical Dementia Rating (CDR) scale of 0.5 in the memory category, objective memory loss as demonstrated by scores on the Logical Memory subtest of the Wechsler Memory Scale-Revised, and a score between 24 and 30 on the Mini-Mental State Exam (MMSE).

Participants were randomized into either a moderate to high-intensity aerobic exercise training (AET) or active stretching/toning (ST) assignment. Exclusion criteria included a diagnosis of AD or other types of dementia or a diagnosis of a major neurological, vascular, or psychiatric disorder. Participants with a history of regular exercise in the last 2 years, body mass index ≥35 kg/m^2, sleep disorders including clinically diagnosed or self-reported sleep apnea, uncontrolled hypertension, diabetes, and a history of smoking within the past 2 years were excluded from the study. Patients who spent >90 minutes of moderate-to-high intensity PA, as assessed by 1 week of monitoring with an Actial accelerometer (Actical, Philips Respironics, USA), were also excluded. Further information about the inclusion/exclusion criteria is located at ClinicalTrials.gov: NCT01146717 and in previous publications (16, 17).

Because we found no difference in lymphocyte populations in the blood and CSF between patients that underwent ST and AET, ST and AET cohorts were pooled into a PA group (Table 1). Nineteen subjects at baseline, and 18 subjects after PA, had blood draws. Fourteen subjects at baseline, and 13 subjects after PA, had lumbar punctures. Sixteen subjects at baseline, and 17 subjects after PA, underwent both cognitive testing and immunophenotyping in the blood. Only 5 aMCI (2 from the ST group, 3 from the AET group) participants had pre- and post-PA sample collection of peripheral blood, and 3 subjects (1 from the ST group, 2 from the AET group) did not have 12-month sample collection, so 6 month samples were used. Forty-one patients underwent PET imaging at baseline, and 12 patients had post-PA imaging.

Table 1. Demographics- cognitive testing and immunophenotyping cohort

	Pre-PA group (n=16)	Post-PA group (n=17)	Mann-Whitney (p value)
Age (55-76)	64.1 (6.0)	65.4 (6.6)	0.54
Race (% Caucasian)	87.5	88.2	0.66
Gender (% Female)	56.2	52.9	0.99
Education (12-18)	15.7 (2.4)	16.0 (2.4)	0.62

Mean (Standard deviation)

Aerobic Exercise Training (AET): Exercise dose and intensity for the AET cohort was determined for each participant's fitness level by assessing the peak oxygen uptake (VO_2) and progressively increasing exercise intensity as individual's adapted to previous weeks of AET. During the initiation phase of AET, participants underwent three 25-30 minute aerobic exercise sessions per week at an intensity of 75-85% maximal heart rate as measured during peak VO_2 at baseline. By week 11 of the intervention, participants had three to four 30-35 minute exercise sessions weekly. On weeks in which participants performed 3 exercise sessions, participants also performed a high intensity exercise session of 30 minutes of brisk uphill walking (85-90% maximal heart rate). By week 26, participants underwent four to five 30-40 minute exercise sessions per week, including two high intensity sessions. During each session, participants had a 5 minute warm-up and 5 minute cool-down. Any form of aerobic exercise was permitted as long as appropriate dose and intensity of training was achieved, as assessed by heart rate during the exercise session by a heart rate monitor watch (Polar RS400, Polar Electro, USA). The AET protocol met the national standards for physical activity guidelines for older adults. Our previous studies found that AET significantly improves cardiorespiratory fitness in sedentary individuals over the age of 65 (19).

ST: The control ST cohort underwent a stretching/toning regimen that focused on the upper arm and lower body with the same frequency and duration as described in the AET protocol above. At week 19, we introduced a second, more advanced set of full body stretching. At week 26, patients began a set of low resistance Theraband exercises focusing on strengthening the upper and lower body. Participants were required to keep

their heart rate below 50% of maximal heart rate as measured by heart monitor watch during each ST session. By using our ST cohort as an active control group we ensured that participants received similar attention from, and interactions with, investigators as those randomized to the AET group.

Immune cell collection and analysis: Biosample collections were performed through The University of Texas Southwestern Medical Center Alzheimer's Disease Center using established protocols (15). Collection was generally performed during morning visits and processing within 60 minutes of collection. Peripheral blood mononuclear cells (PBMC) and cerebrospinal fluid (CSF) immune cells were isolated by centrifugal Ficoll-based separations previously described (16). CSF samples tinged red were excluded from further analysis. PBMCs and CSF cells were stained with fluorescent antibodies and data acquired via flow cytometry. Our multi-parameter antibody panel consisted of CD45 (APC-Cy7), CD4 (PE-Cy7), CD8 (APC), CD19 (PerCPCy5.5 or Brilliant Violet 421), CD27 (FITC-A), and CD138 (PE-A) antibodies (BD Biosciences, San Jose, CA, USA). Five patients at baseline and 3 PA subjects (ST n=1, AET n=2) did not have CD3 staining. No live/dead stain was used as cells were rapidly processed after collection. Gating strategies were previously published (16). Flow cytometry data were analyzed (Flowjo; Tree Star) and normalized to the $CD45^+$ cell gate to compare over time and across samples. CSF cell analysis included all cells collected, with no minimum cell number required to perform flow cytometry.

PET imaging: aMCI patients received a bolus of 10-mCi ^{18}F-florbetapir 30-min prior to positioning in a Siemens ECAT HR PET scanner for a 10-min emission and 10-min transmission scan, as previously described in detail (16). Fifty minutes after tracer injection, a 5-min PET emission scan and a 7-min transmission scan were acquired. Every PET image was normalized spatially to ^{18}F-florbetapir uptake template using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK) and MATLAB scripts (Mathworks Inc., Natick, MA) and inspected for quality. Standardized uptake value ratio (SUVR) was computed and compared to mean cerebellar uptake as a brain reference. The mean cortical SUVR was the average of: posterior and anterior cingulate, precuneus, temporal, dorsolateral prefrontal, orbitofrontal, parietal, and occipital SUVRs (17).

Neurocognitive tests: aMCI diagnosis was based on Petersen criteria (18), as modified from Alzheimer's Disease Neuroimaging Initiative (ADNI) project (http://adni-info.org). Cognitive testing (Table 2) included well validated measures of attention/concentration (Digit Span Forward and Backwards), processing speed (Trails A), memory (California Verbal Learning Test; CVLT), Wechsler Memory Scale-Logical Memory Immediate Recall); LMIR verbal fluency (Letter Fluency, Semantic Fluency); LMIR, and executive functioning (Trails B, Stroop Color Word Interference Test). All tests but LMIR (raw score) and CVLT (t-score) and with all cognitive function tests, higher scores reflect better cognitive function.

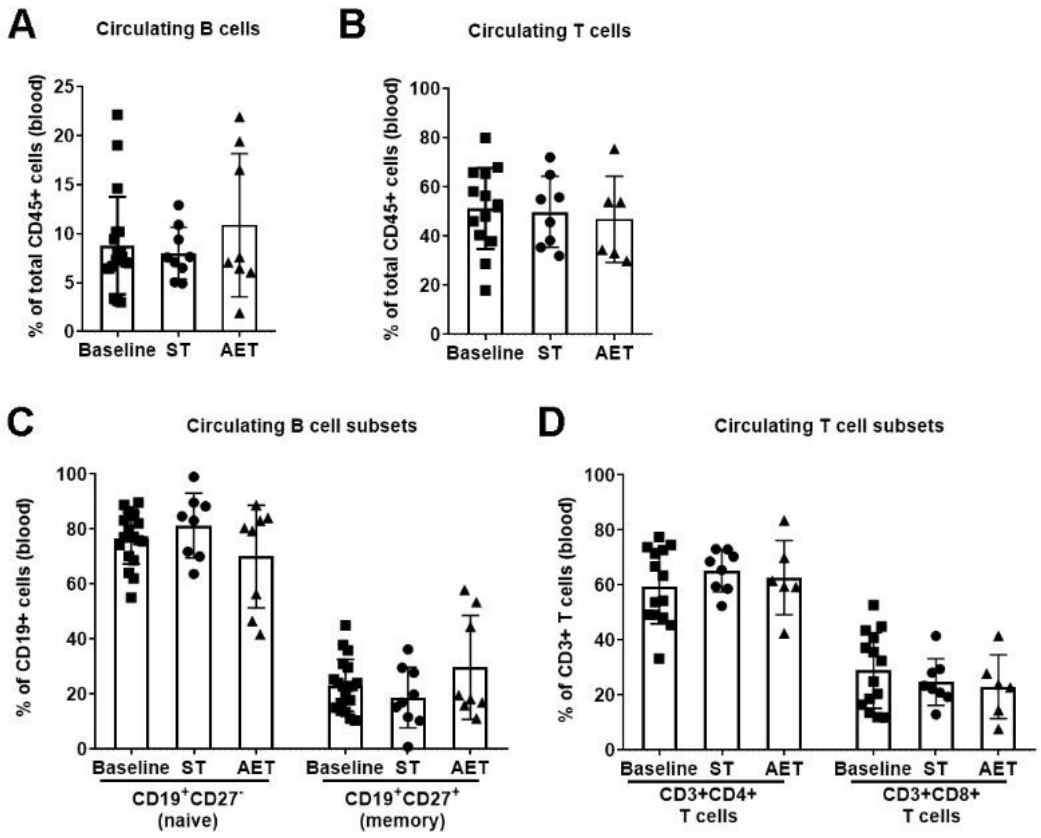


Figure 1. Aerobic exercise training and stretching/toning exert minimal effects on adaptive immune cell populations in aMCI patients. General (A) B cell ($CD19^+$) and (B) T cell (CD3+) populations in the blood do not differ between sedentary baseline (squares; n=19) and individuals in the stretching/toning (ST; circles; n=9) and aerobic exercise training (AET; triangles; n=8) interventions. There is also no difference for circulating (C) B cell subsets (baseline, n=19; ST, n=9; AET, n=8) and (D) T cell subsets in the blood. 3 individuals were excluded from overall T cell and T cell subset quantification due to insufficient $CD3^+$ staining.

Table 2. Summary of cognitive scores by group					
	Pre-PA group (n=16)		Post-PA group (n=17)		Mann-Whitney (p value)
Cognitive tests by domain	**Mean**	**SD**	**Mean**	**SD**	
Attention and Concentration					
Digit Span Forward	8.07	2.58	8.35	2.37	0.786
Digit Span Backwards	6.2	2.51	6.65	2.45	0.565
Processing Speed					
Trails A	11.94	2.14	13.38	1.85	***0.019****
WAIS-R Coding	11.5	2.42	12.06	2.32	0.579
Memory					
Logical Memory Immediate Recall	11.88	1.36	12.76	2.44	0.266
CVLT Total (t-score)	49.19	9.88	49.35	12.48	0.715
Verbal Fluency					
Letter Fluency	10.25	1.92	12.13	3.56	0.145
Semantic Fluency	9.88	3.42	12.73	3.52	***0.043****
Executive Function					
Trails B	11.69	2.06	12.06	2.25	0.672
Color Word Interference Test	10.47	1.88	11.13	2.42	0.238
CVLT, California Verbal Learning Test; *p<0.05 with significant values bolded					

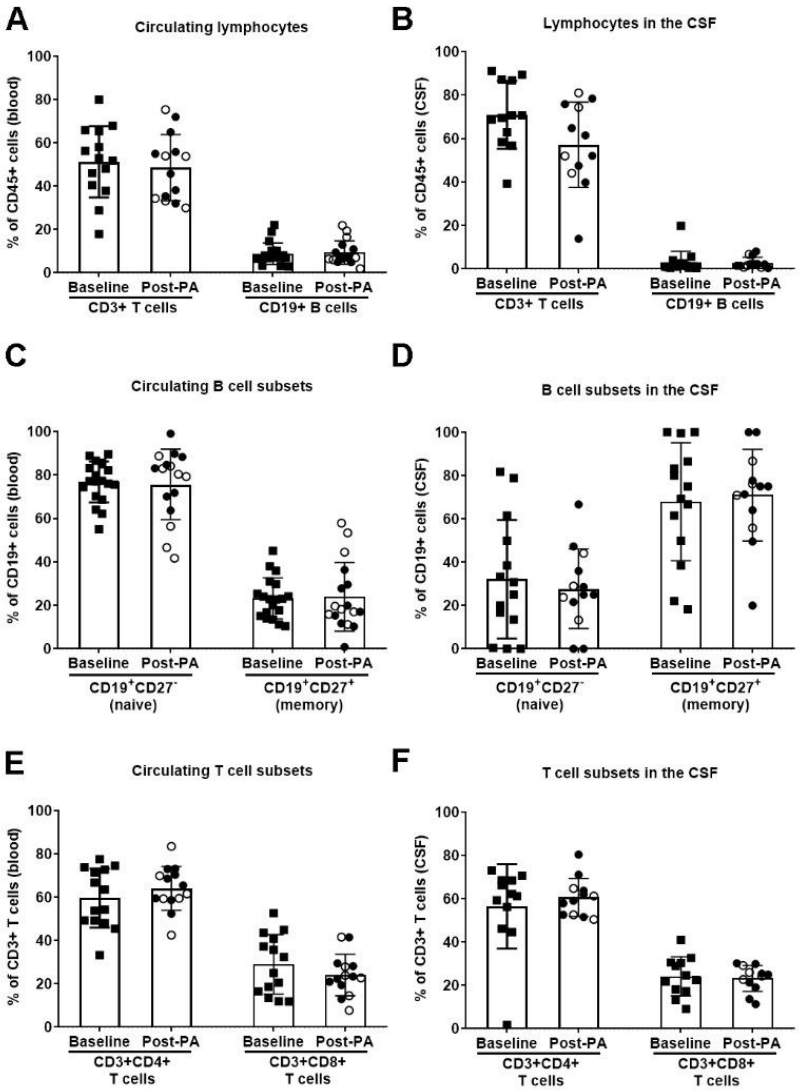


Figure 2. Physical activity does not alter adaptive immune profiles in aMCI patients. General T cell ($CD3^+$) and B cell ($CD19^+$) populations in (A) blood or (B) cerebrospinal fluid (CSF) do not differ between sedentary baseline (squares) and physical activity (PA) groups, including individuals in the stretching/toning (closed circles) and aerobic exercise training (open circles) interventions. There is also no difference for B cell subsets in the (C) blood and (D) CSF, as well as T cell subsets in the (E) blood and (F) CSF..

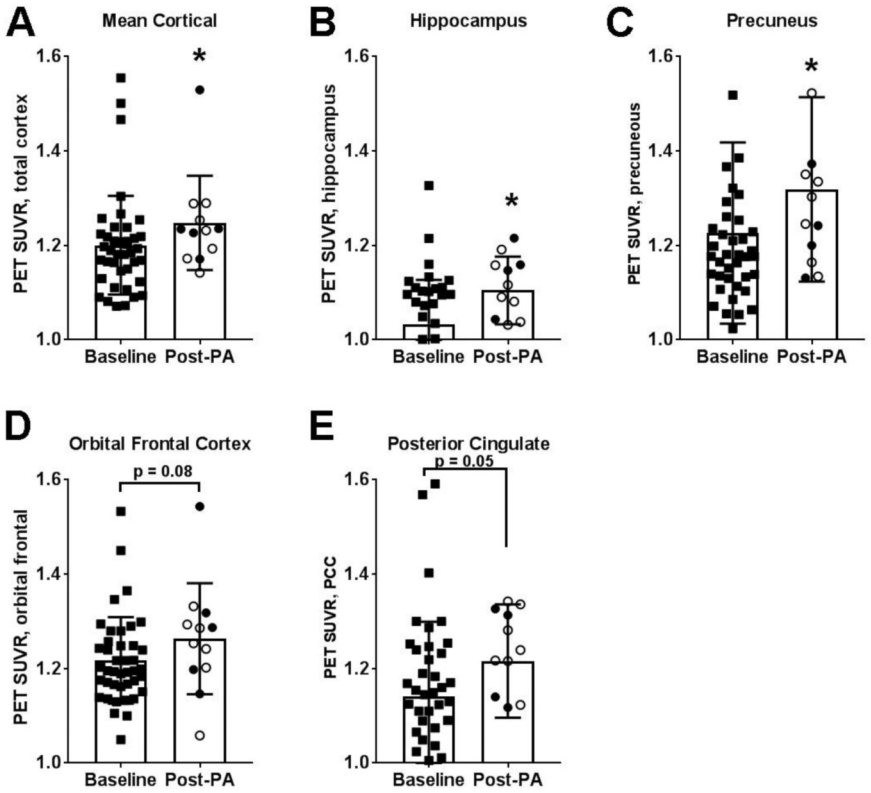


Figure 3. Overall amyloid burden increased between sedentary baseline and after 6-12 month of physical activity. Standardized uptake value ratio (SUVR) for PET imaging of 18F-florbetapir (amyloid burden) demonstrated a significant increase in Aβ load for (A) total brain, (B) hippocampus, and (C) precuneous cortices between sedentary baseline (squares; n=41) and physical activity (PA) groups, including individuals in the ST (closed circles; n=5) and aerobic exercise training (open circle; n=7) interventions. There were also trends for increased Aβ in the (D) orbital frontal and (E) posterior cingulate cortices. *p<0.05 vs Baseline.

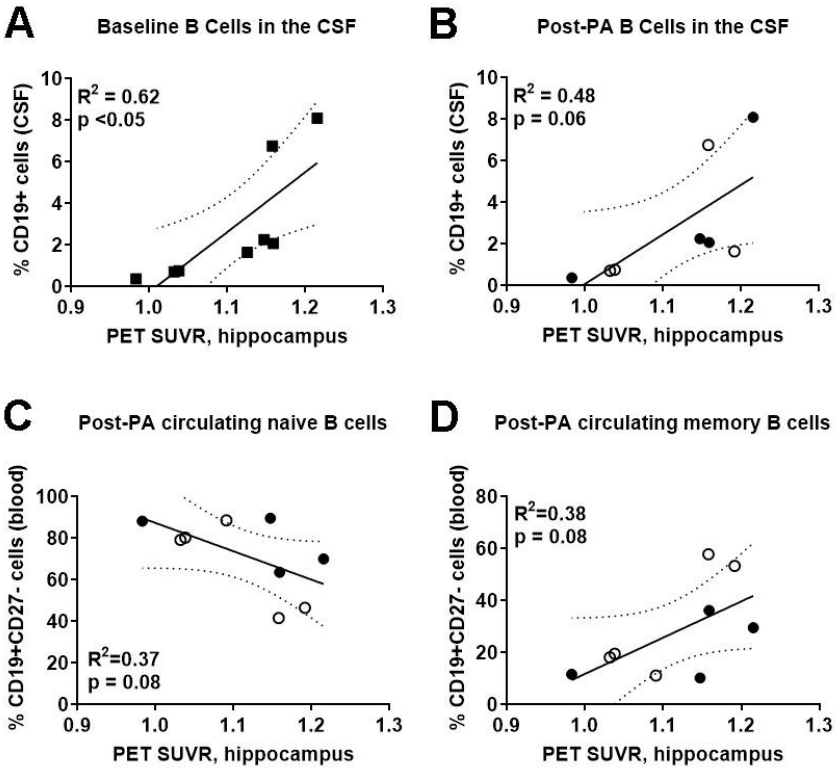


Figure 4. Higher levels of CSF-localized B cells and circulating memory B cells associate with increased hippocampal Aβ deposition. Standardized uptake value ratio (SUVR) for PET imaging of 18^{F}-florbetapir (amyloid burden) in the hippocampus associated with higher $CD19^{+}$ B cells in the cerebrospinal fluid (CSF) for both (A) sedentary baseline (squares; n=8) and (B) physical activity (PA) groups, including individuals in the ST(closed circles; n=4) and AET (open circle; n=4) interventions. There were concomitant trends for (C) decreased circulating naïve B cells and (D) increased circulating memory B cells after PA.

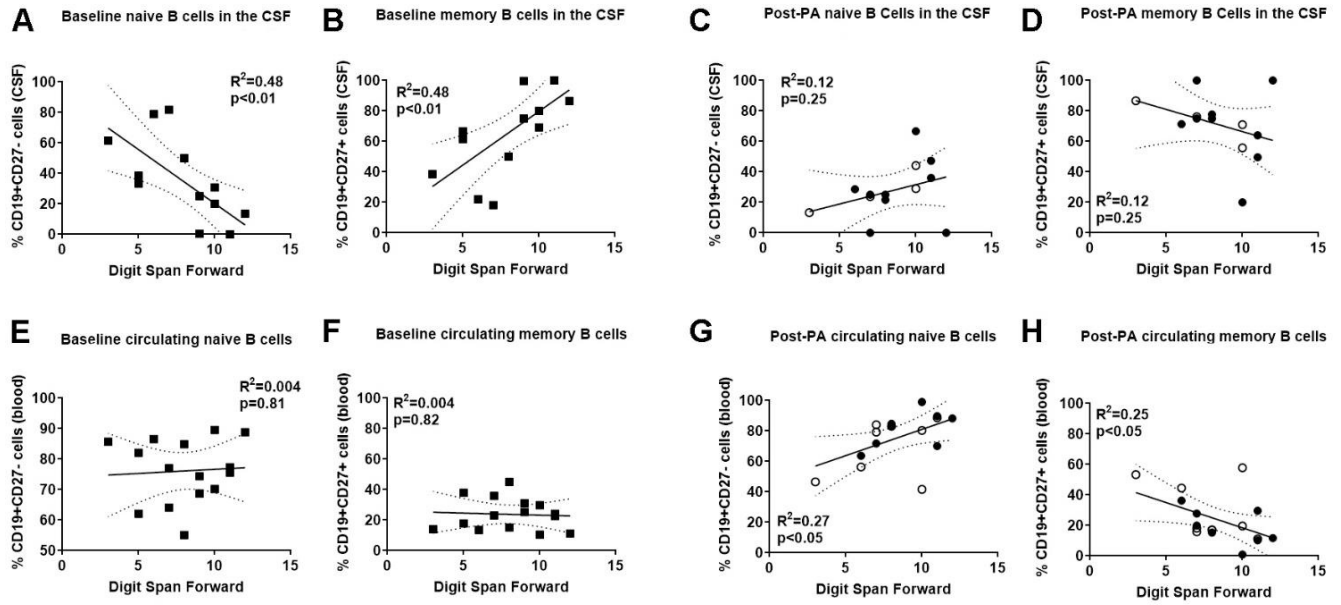


Figure 5. Shifts from naïve to memory B cell populations reflect neurocognitive function. (A-D) Sedentary baseline (squares; n=15) aMCI subjects with better attention and concentrations (i.e. higher numbers, digital span forward test) exhibit (A) lower naïve B cells and (B) more memory B cells in the cerebrospinal fluid (CSF). This relationship in the CSF is lost in post-physical activity (PA) groups for (C) naïve and (D) memory B cells. Individuals in the ST (closed circles; n=9) and AET (open circles; n=4) interventions are identified. (E-H) Circulating B cell populations in the same subjects show no correlation at baseline for (E) naïve and (F) memory B cell populations. However, post-PA groups exhibit (G) higher naïve B cells and (H) lower circulating memory B cells with improved attention and concentration.

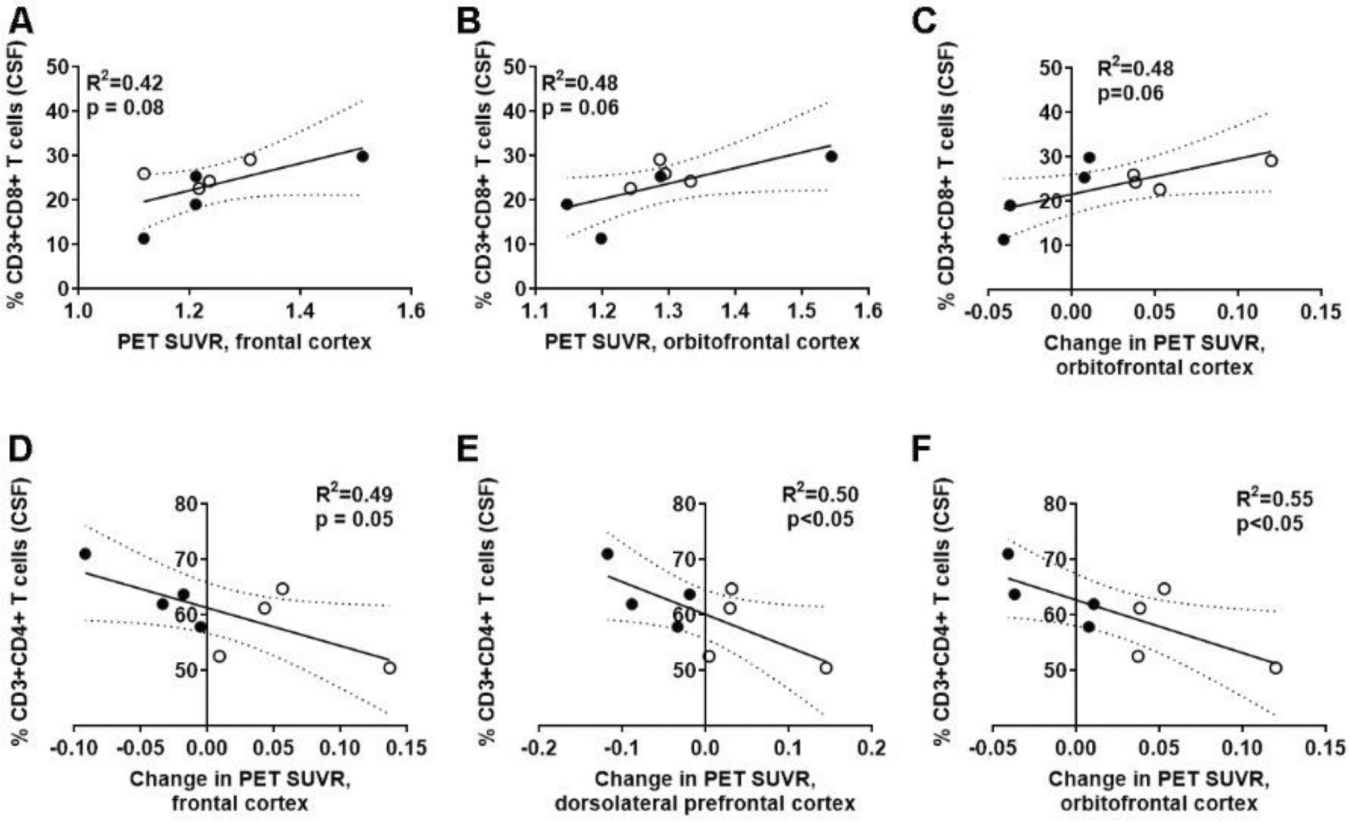


Figure 6. Higher levels of CSF-localized CD8 T cells, and lower CD4 T cells associate with increased Aβ deposition after physical activity. (A-C) Standardized uptake value ratio (SUVR) for PET imaging of 18^{F}-florbetapir (amyloid burden) associated higher $CD8^{+}$ T cells in the cerebrospinal fluid (CSF) in both (A) frontal and (B) orbitofrontal cortices after PA. Individuals in the ST (closed circles; n=4) and AET (open circles; n=4) interventions are identified. (C) Increased CD8 T cells also associated with increased Aβ deposition. (D-F) There were concomitant decreases for CD4 T cells in (D) frontal, (E) dorsolateral prefrontal, and (F) orbitofrontal cortices with increased Aβ deposition when comparing baseline and post-PA PET imaging.

Statistical analysis: All data are reported as mean ± standard deviation, with statistical significance set a *priori* at $p<0.05$ for all tests and trending values were defined as $p\leq0.06$. Kruskal-Wallis tests were performed to compare immune populations between baseline, AET, and ST cohorts. Mann-Whitney tests were performed to compare the baseline and the overall PA sample (composed of both AET and ST groups) and to compare age, education level, CDR, and cognitive results between groups as appropriate. Fisher's Exact tests were performed to see if sex or race differed between groups. Linear regressions were performed to examine the relationships between adaptive immune populations, brain Aβ burden, and cognitive domains. Multiple comparison correction was not performed for this exploratory study and all statistical analyses were performed using GraphPad Prism (La Jolla, CA).

RESULTS

Physical activity does not modulate frequency of B and T cells in aMCI patients

To determine if PA impacted adaptive immunity in the periphery and/or central nervous system (CNS), we analyzed B and T cell subsets in the blood and CSF isolated from a subset of aMCI patients at baseline (n=19) and subsets of aMCI patients after either AET (n=8) or ST (n=9) intervention. Overall, $CD19^+$ B cells and $CD3^+$ T cells in the CSF (data not graphed) and blood (Fig. 1A-B) did not differ between interventions. Furthermore, there was no difference in any circulating B or T cell subset, including naïve B cells, memory B cells, $CD4^+$ T cells, and $CD8^+$ T cells (Fig. 1C-D). Given no observable differences in the distribution of B and T cells in the blood and CSF, AET and ST cohorts were pooled. After PA, B and T cells (and their respective subsets) did not differ from baseline in either CSF or blood (Fig. 2). Our preliminary data from this pilot sample of aMCI participants suggests that the distribution of adaptive immune cells in the CSF and blood do not change after an extended period of PA.

B cells were associated with hippocampal Aβ burden

To understand the relationship between adaptive immunity and Aβ burden, we first examined whether PA altered Aβ burden in multiple regions of the brain. In aMCI patients, we identified a significant increase in mean cortical Aβ burden ($p<0.05$) and Aβ deposition in the hippocampus ($p<0.05$) and precuneus ($p<0.05$) post-PA (Fig. 3). There was also a trending increase in Aβ burden in the posterior cingulate ($p=0.05$; Fig. 3E). Next, we sought to determine if there were correlations between Aβ burden and overall B cell populations in the CSF and blood both before and after PA. For sedentary aMCI patients prior to PA, those with higher Aβ burden in the hippocampus exhibited higher B cell representation in the CSF ($R^2=0.62$; $p<0.05$; n=8; Fig. 4A). This relationship persisted after PA; more B cells in the CSF were associated with greater hippocampal Aβ deposition ($R^2=0.48$; $p=0.06$; Fig. 4B). There was no correlation between hippocampal Aβ deposition and overall B cells in the blood at baseline ($R^2=0.024$, $p=0.69$) and after PA ($R^2=0.025$, $p=0.68$).

Finally, we examined correlations between Aβ burden and particular subsets of B cells. At baseline and after PA, neither CSF-derived naïve nor memory B cell populations were associated with Aβ burden in the hippocampus (naïve-$R^2=0.31$; memory-$R^2=0.33$). Similarly, there were no correlations between either subset in the blood (naïve-$R^2=0.25$; memory-$R^2=0.18$) at baseline. After a one-year PA intervention, however, participants with increased hippocampal Aβ deposition had fewer naïve B cells ($R^2=0.37$; $p=0.08$) and more memory B cells in the blood ($R^2=0.38$; $p=0.08$), signifying a shift, albeit non-significant, in B cell phenotype (Fig. 4C-D). Other brain regions examined by PET (anterior and posterior cingulate, dorsolateral prefrontal cortex, frontal cortex, orbitofrontal cortex, precuneus, and temporal lobe) were not associated with changes in B cells or associated B cell subsets.

Peripheral B cell subsets differentially associated with cognitive test results

We examined whether there was a relationship between immune cell subsets and cognitive function, and if this differed after PA relative to baseline. First, we examined whether PA affected neurocognitive functioning in our pooled PA sample. Following PA, aMCI subjects performed similarly across most tests but showed slight but statistically significant improvements on tests of processing speed and semantic fluency (Table 2). The frequency of overall $CD19^+$ B cells in the CSF did not correlate with any measures of cognitive function at either baseline or after PA. Circulating $CD19^+$ B cells correlated with performance on only one measure of processing speed (Table 3), with fewer B cells in the blood associated with higher scores on the Trails A task ($R^2=0.45$; $p<0.01$). However, the inverse was true after PA as a higher frequency of circulating B cells correlated with better performance ($R^2=0.30$; $p<0.05$; data not graphed).

Subset-specific relationships between naïve and memory B cells and neurocognitive functioning were also examined. First, fewer naïve B cells ($R^2=0.48$; $p<0.01$) and more memory B cells ($R^2=0.48$; $p<0.01$) in the CSF associated with higher scores on measures of attention and concentration (Fig. 5A-B), as well as verbal fluency (Table 3) at baseline. After PA, all correlation between CSF-localized B cell subsets and performance on any neurocognitive task was lost (Fig. 5C-D). In the blood, baseline levels of circulating B cell subsets did not correlate with any measure of cognitive function (Fig. 5 E-F; Table 3). However, after PA, attention and concentration scores correlated with the frequency of B cell subsets in the blood. Specifically, a higher frequency of naïve B cells ($R^2=0.27$; $p<0.05$) and lower frequency of memory B cells in the blood ($R^2=0.25$; $p<0.05$) associated with higher attention and concentration scores (Fig. 5 G-H). Together, this shows that naïve and memory B cells and cognitive correlates were minimal and varied based on the tissue and time point examined.

CSF-localized $CD4^+$ T cells decrease with higher brain Aβ deposition after physical activity

Again, we investigated both the amyloid imaging at either baseline or post-PA, as well as the change of amyloid deposition over one year within subjects with both baseline and post-PA PET imaging. Investigation into CSF-derived T cell subsets and PET imaging showed that at baseline, there were no

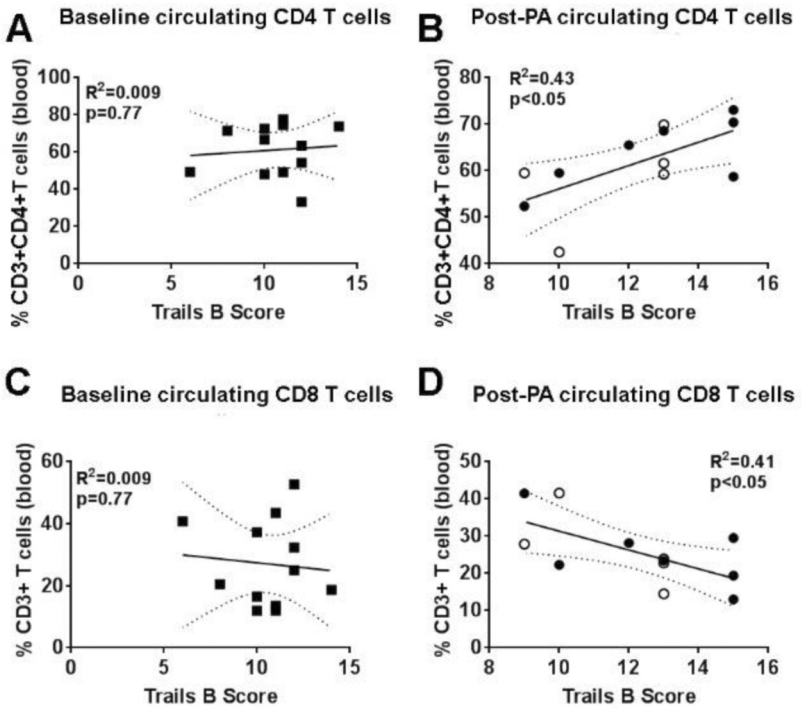


Figure 7. Circulating T cell populations reflect neurocognitive function after long-term physical activity. (A) Sedentary baseline (squares; n=12) aMCI subjects with better executive function (i.e. higher numbers = better cognitive function, Trails B) exhibit no trend for either circulating (A) $CD4^+$ T cells or (C) $CD8^+$ T cells. Physical activity (PA) group, including individuals in the stretching/toning (closed circles; n=7) and aerobic exercise training (open circles; n=5) interventions exhibit opposite patterns for T cell subsets in the circulation, with improved executive function associated with more (B) $CD4^+$ T cells and fewer (D) $CD8^+$ T cells in the blood.

Table 3. Linear regression of adaptive lymphocyte populations vs. neurocognitive outcomes

		blood (R^2 values)						CSF (R^2 values)					
		CD3+ T cells	CD4+ T cells	CD8+ T cells	CD19+ B cells	Naïve	Memory	CD3+ T cells	CD4+ T cells	CD8+ T cells	CD19+ B cells	Naïve	Memory
Attention and Concentration													
Digit Forward	**Baseline**	0.00	0.21	0.29	0.00	0.00	0.00	0.02	0.05	0.11	0.02	**↓0.48****	**↑0.48****
	Post	0.14	0.00	0.00	0.02	**↑0.27***	**↓0.25***	0.00	0.06	0.01	0.05	0.12	0.12
Digit Backwards	**Baseline**	0.02	0.01	0.01	0.00	0.02	0.02	0.00	0.04	0.01	0.03	0.05	0.04
	Post	0.00	0.03	0.00	0.00	**↑0.25***	**↓0.25***	0.00	0.12	0.12	0.02	0.00	0.01
Processing Speed													
Trails A	**Baseline**	0.11	0.01	0.00	**↓0.45****	0.16	0.16	0.00	0.04	0.04	0.00	0.09	0.07
	Post	0.32	0.14	**↓0.35***	**↑0.30***	0.10	0.12	0.23	0.09	0.28	0.33	0.02	0.01
WAIS-R Coding	**Baseline**	0.07	0.11	0.10	0.05	0.08	0.09	0.00	0.05	0.05	*0.12*	0.01	0.02
	Post	0.13	0.19	0.27	0.07	0.21	0.23	0.28	0.00	0.01	0.11	0.03	0.05
Memory													
LMIR	**Baseline**	**↓0.40***	0.00	0.00	0.03	0.00	0.00	0.31	0.03	0.03	0.04	0.01	0.01
	Post	0.07	0.05	0.02	0.00	0.03	0.03	0.06	0.00	0.05	0.14	0.01	0.01
CVLT Total (t-score)	**Baseline**	0.01	0.13	0.06	0.15	0.00	0.00	0.01	0.12	0.00	0.05	0.03	0.02
	Post	**↓0.36***	0.02	0.00	0.01	0.11	0.10	0.08	0.12	0.08	0.03	0.00	0.00
Verbal Fluency													
Letter Fluency	**Baseline**	0.13	**↓0.47****	**↑0.53****	0.07	0.05	0.05	0.05	0.14	0.12	0.11	**↓0.40***	**↑0.41***
	Post	0.28	0.10	0.01	0.02	0.04	0.03	0.01	0.00	0.15	0.00	0.12	0.08
Semantic Fluency	**Baseline**	0.04	**↓0.51****	**↑0.59****	0.16	0.02	0.03	0.10	0.04	0.00	0.03	0.06	0.04
	Post	**↓0.33***	0.01	0.07	*0.20*	0.12	0.12	0.14	0.00	0.11	0.30	0.04	0.04
Executive Function													
Trails B	**Baseline**	0.13	0.01	0.01	0.01	0.01	0.04	0.03	0.09	0.06	0.00	*0.22*	0.21
	Post	0.16	**↑0.43***	**↓0.41***	0.04	0.11	0.12	0.20	0.02	0.00	0.01	0.09	0.11
CWIT	**Baseline**	0.13	0.01	0.01	0.04	0.01	0.01	0.03	0.00	0.03	0.07	0.02	0.02
	Post	0.11	0.19	0.22	0.06	0.06	0.06	**↑0.39***	0.03	0.00	0.02	0.03	0.05

CWIT: Color Word Interference Test; LMIR, Logical memory Immediate Recall; up arrows indicate positive correlation, down arrows indicate negative correlation; *p<0.05; **p<0.01

correlations with Aβ burden lymphocyte profiles. After one year of PA, however, there was a trend for higher frequencies of $CD8^+$ T cells in the CSF to be associated with increased Aβ burden in the orbitofrontal cortices (R^2=0.48; p=0.06; Fig. 6B). Furthermore, a greater number of CD8+ T cells in the CSF correlated with an increase in Aβ burden in the orbitofrontal cortex over time (R^2=0.48; p=0.06; Fig. 6C), although this did not reach statistical significance. Peripheral $CD8^+$ T cells did not correlate with either Aβ deposition or the change in deposition over the one year for the aMCI patients with pre-post PET imaging and post-PA immunophenotyping. CSF-localized $CD4^+$ T cells were only associated with changes in Aβ burden over time. Specifically, decreased frequency of $CD4^+$ T cells in the CSF correlated with the increased Aβ deposition in brain regions associated with executive functioning (20), namely the frontal cortex (R^2=0.49; p=0.05; n=8; Fig. 6D), dorsolateral prefrontal cortex (R^2=0.50; p<0.05; Fig. 6E), and orbitofrontal cortex (R^2=0.55; p<0.05; Fig. 6F). Peripheral circulating $CD4^+$ T cells were not associated with changes in any of these regions. Together, this shows a CSF-specific relationship between T cell subsets and Aβ deposition, similar to our previous results at baseline (16), and confirms that PA does not affect this relationship.

Improved cognitive function after physical activity associated with increased circulating $CD4^+$ T cells

Given the association with $CD4^+$ and $CD8^+$ T cells with Aβ burden in regions associated with executive function, we sought to examine if executive functioning, or other cognitive domains, were affected by the frequency of T cells in the CSF and blood. At baseline, $CD3^+$ T cells in the CSF did not associated with cognitive function. After PA, higher levels of CSF-derived T cells associated with better scores on one measure of executive function (Table 3), however CD3 is a pan-T cell marker and neither the $CD4^+$ nor $CD8^+$ subpopulations in the CSF reflected this correlation (Table 3). Baseline circulating levels of $CD3^+$ T cells in the blood associated with higher Logical Memory scores (Table 3). After PA, fewer $CD3^+$ T cells in the periphery were associated with higher scores on memory verbal fluency tasks (Table 3).

Like B cells after PA, CSF-derived $CD4^+$ and $CD8^+$ T cell subsets did not associate with cognitive outcomes, but instead effects were limited to the circulating lymphocyte populations. In stark contrast to the complete lack of correlation at sedentary baseline (R^2=0.009; p=0.77; Fig. 7A, Table 3), higher levels of circulating $CD4^+$ T cells associated with improved executive function after PA (R^2=0.43; p<0.05; Fig. 7B). Conversely, the frequency of $CD8^+$ T cells, which also had no correlation with executive functioning or processing speed at baseline (R^2=0.009; Fig. 7C), declined in the blood of subjects displaying higher executive functioning and processing speed scores (R^2=0.41; p<0.05; Fig. 7D) after PA. Together, these data indicate that peripheral $CD4^+$ and $CD8^+$ T cells differentially associate with neurocognitive functioning in aMCI patients after PA.

DISCUSSION

Defining MCI

As mentioned above, MCI is a neurocognitive disorder distinguished by a cognitive decline greater than typically expected for normal aging. Individuals with MCI are still able to perform all of their daily activities, but they may take longer and/or use adaptive strategies (e.g. memory aids) to successfully complete their day-to-day tasks (21). Based on which cognitive domains are affected, individuals with MCI will be classified as aMCI or non-amnestic MCI (naMCI). aMCI refers to cognitive impairment solely in the domain of learning and memory, including difficulty in the retrieval of recently stored information. Conversely, naMCI refers to impairments in one or more cognitive domains, excluding learning and memory. Approximately 5-10% of individuals with MCI progress to dementia annually (22, 23). Furthermore, those with a diagnosis of naMCI are more likely to progress toward other dementias, such as Lewy body dementia, vascular dementia, or frontotemporal dementia, whereas patients with aMCI are at higher risk of developing AD (21, 24-26). Known risk factors for MCI include age, male sex, presence of APOε4 allele, and family history of cognitive impairment (27-30). Chronic conditions including hypertension, hyperlipidemia, coronary artery disease, stroke, osteoarthritis, chronic obstructive pulmonary disease, depression, traumatic brain injury, and diabetes mellitus have been identified as risk factors for MCI (31-35). Sedentary behavior, both cognitively and physically, is also a proposed risk factor for MCI (36, 37), underscoring the importance of exercise as a potential therapeutic intervention for MCI patients.

Immune alterations in MCI patients

A wide range of mechanisms contribute to MCI pathology. These include structural changes in the brain, accumulation of Aβ and neurofibrillary tangles, declining neuroplasticity, dysfunctional cholinergic and serotonergic systems, and increased oxidative stress (38). In addition to these mechanisms, recently we and others reported immune changes in the periphery and CNS of aMCI subjects (15, 16, 39). As healthy individuals age, leukocytes become immunosenescent (40-42) and B and T cell function declines (40). B and T cells are two components of the adaptive immune system. B cells serve two primary functions: secretion of antibodies and antigen presentation (43). After encountering antigens, naïve B cells become short-lived plasmablasts or memory B cells (43). Memory B cells produce very specific antibodies upon re-exposure to a familiar antigen that induced their formation. B cells can present antigens to other cells including $CD4^+$ T cells (i.e. helper T cells) that recognize antigens and secrete large quantities of cytokines (43). $CD8^+$ T cells, also known as cytotoxic T cells, recognize particular antigens and induce apoptosis in foreign cells (43). Naïve B and T cells decline in the periphery with age, reducing antibody production and responses to mitogen stimulation, respectively, with a simultaneous increase in anergic memory T and B cells (40, 44). Furthermore, a low-grade systemic inflammation develops with age, leading to greater secretion of pro-inflammatory cytokines and decreased anti-inflammatory regulatory immune cells (42, 44). MCI patients present with greater immunosenescence and inflammation, likely con-

tributing to disease pathogenesis and progression toward dementia (45).

While the data are not conclusive, there is significant evidence suggesting that pro-inflammatory cytokines are increased in MCI (46). Some studies report higher serum/plasma levels of inflammatory cytokines (e.g. IFN-γ, TNF-α) and chemokines (46). In the CSF, fewer cytokines and chemokines have been examined, but MCP-1 (i.e. CCL2), a chemokine that promotes leukocyte recruitment, was increased in the CSF of MCI patients while TGF-β, an anti-inflammatory cytokine, was downregulated in the CSF (46). Interestingly, some inflammatory factors also associate with the progression of MCI to AD. High levels of soluble CD40 (sCD40) at baseline correlate with the risk of progression from MCI to AD over the course of 4-7 years (47). In both MCI-AD and control cases, higher levels of plasma sCD40 were associated with lower MMSE scores, representing global cognitive decline (47). sCD40 interferes with B cell-T cell interactions and can suppress the development of memory B cells, which could contribute to the dysregulation of the ratio of naïve to memory B cells that we see in our patients (48, 49). Another promising predictor of progression from MCI to AD is the soluble TNF-α receptor, sTNFR (50). MCI patients that progressed to AD had higher levels of sTNFR1 than patients that did not progress (50). While further research is needed, it is possible that alterations in sCD40 and sTNFR1 may be useful immunological biomarkers to determine if an MCI patient is likely to progress to AD, which could allow for early interventions such as exercise.

In addition to cytokines, researchers examined whether leukocyte populations are altered in MCI patients. Differences in T cell profiles in MCI patients include diminished $CD3^+$ T cells in the blood of MCI patients relative to healthy controls (51). In the CSF, activated $CD4^+$ T cells were increased in both patients with MCI and mild AD relative to healthy controls, while activation of $CD8^+$ T cells was associated with parahippocampal structural damage and cognitive deficits (52). In our sedentary cohort, we found that Aβ burden was negatively associated with CSF-derived $CD4^+$ T cell populations, with fewer $CD4^+$ T cells correlating with greater Aβ deposition over time (53). Therefore, increasing $CD4^+$ T cells in MCI patients may be playing a beneficial role in the CNS, particularly as aMCI patients have more anti-inflammatory regulatory T cells (Tregs) (54, 55). Unfortunately, our immune panel did not specifically identify Tregs, but this suggests that decreases of potentially beneficial $CD4^+$ T cells, and concomitant increases of potentially pathogenic $CD8^+$ T cells, contribute to aMCI pathology and deterioration of cognitive functioning. Further research is necessary to improve our understanding of the functional role of these T cell subsets in aMCI patients and during the progression to AD.

Less is known about how B lymphocytes affect, alter, and/or support cognition. Magaki et al. reported that overall B cells in the periphery did not significantly differ between aMCI and healthy controls (51). However, in our prior study we found subset-specific changes in B cell populations in sedentary aMCI patients as naïve B cells were decreased and memory B cells were increased in APOε4 carriers with aMCI (16), who are at a significantly greater risk of developing AD (53). Given that the switch from naïve to memory phenotype is observed in healthy elderly individuals (40), our findings suggest that aMCI-induced immune changes are potentially a more severe form of immunosenescence that naturally occurs with aging.

Aβ and the adaptive immune response during MCI

One of the hallmark features used to predict the progression from MCI to AD is the accumulation of Aβ in the brain (2, 3). Aβ deposition is believed to trigger the cascade of events leading to brain atrophy and synaptic dysfunction characteristic of AD (2, 3). In response to Aβ deposition, leukocyte function and phenotype may also be altered. Based on our small cohort of aMCI patients, we found preliminary evidence of a significant relationship between Aβ burden and different lymphocyte populations in aMCI. First, we found that brain Aβ deposition increased in several brain regions over one year despite initiation of PA. These are predictable results given that these regions have previously been identified as sites of early Aβ deposition (56). In MCI patients, greater accumulation of Aβ in the posterior cingulate over the course of 2 years associated with more severe memory impairments and higher rates of progression to AD (57, 58). It is possible, therefore, that the Aβ deposition we observed in those regions was a product of the natural progression of aMCI over time and was not impacted by PA. However, the specific hippocampal Aβ deposition we observed has been less thoroughly characterized in aMCI patients. Greater hippocampal atrophy and hyperexcitability is associated with worse cognitive decline and progression to AD (59) and thus hippocampal Aβ may be a potentially interesting therapeutic target.

In our study, we found significant correlations between B and T cells and brain Aβ burden during aMCI. CSF-localized $CD8^+$ T cells positively correlated with Aβ beta burden in the orbitofrontal cortex after PA. Conversely, $CD4^+$ T cells decreased in aMCI patients that had greater accumulation of Aβ over time in the frontal cortex, dorsolateral prefrontal cortex, and orbitofrontal cortex. This inverse relationship between $CD4^+$ and $CD8^+$ T cells in the CSF suggests that these cell types may be playing different roles in response to Aβ in the brain. In AD patients, T cells, in particular $CD8^+$ T cells, increase in the extravascular space in the hippocampus and midfrontal gyrus (60). Similarly, we found that there was an increase in $CD8^+$ T cells in the CSF of MCI patients with higher Aβ burden in frontal cortices, suggesting that $CD8^+$ T cells are pathogenic or elevated in response to the pathology. Previous work found that $CD8^+$ T cell influx into the extravascular space associated with tau pathology but not Aβ burden (60), thus future studies should determine possible mechanisms of action for cytotoxic T cells in the development of dementia.

Our data also suggest that $CD4^+$ T cells have the opposite relationship to Aβ deposition compared to $CD8^+$ T cells. We found that as Aβ burden declined, there were a greater number of $CD4^+$ T cells in the CSF. In a murine model of AD, injections of Aβ-specific $CD4^+$ T cells injected in the CSF migrate into the brain where they target Aβ plaques in the hippocampus and cortex, increase phagocytosis of Aβ by innate

immune cells, and stimulate neurogenesis (61). Another study demonstrated that injecting Aβ-specific $CD4^+$ T cells into the blood decreases Aβ burden and improves behavioral function in mice (62). In patients exhibiting cognitive impairment, higher levels of $CD4^+$ Tregs capable of producing anti-inflammatory cytokines correlated with lower levels of amyloidopathy (63). Thus, the recruitment of $CD4^+$ T cells with increased PA may reflect an activation of an adaptive immune-mediated response to counter progression of the disease.

B cell response to Aβ burden is not as well defined as that of T cells. In aMCI sedentary subjects, decreased overall B cells in the periphery were associated with greater Aβ burden in the precuneus (53), whereas in this study increased CSF-derived B cells were associated with greater Aβ burden in the hippocampus both before and after PA. Both of these suggest a movement from the periphery into the CSF with increased Aβ deposition (or conversely a loss of amyloid clearance (64)). Memory B cells may be a particularly important B cell subset; memory B cells isolated from the blood of mild AD patients have increased expression of CCR5, a pro-inflammatory chemokine receptor that enhances recruitment of lymphocytes to sites of inflammation, such as Aβ plaques (65). At baseline and after PA, increased peripheral memory B cells and decreased naïve B cells associated with greater hippocampal Aβ burden. We also previously found that CSF-derived memory B cells increased as Aβ burden increased in sedentary aMCI patients (53). Further investigations are required to determine the protective or pathogenic potential of B cell subsets in the progression of Aβ deposition.

Cognition and adaptive immune responses

In addition to the accumulation of Aβ and learning and memory deficits, individuals with aMCI may exhibit deficits in other cognitive domains. Inflammation in the CNS and periphery is associated with worse cognition in healthy individuals, particularly in the areas of learning, memory and attention, while elevated peripheral cytokine levels in the blood are related to impaired cognitive performance (66). Additionally, lymphocyte subsets have been associated with neurocognitive functioning (67). In healthy elderly, better cognitive function is associated with lower numbers of $CD4^+$ T cells and higher numbers of naïve $CD8^+$ T cells and B cells. Even when variables such as age, mood, and length of education were controlled for, lower levels of circulating effector $CD4^+$ T cells and higher levels of B cells correlated with better performance on measures of executive function and memory (67).

Previously it has been shown in patients with MCI that T cells were associated with changes in cognitive functioning (52). In particular, CSF-localized, activated $CD8^+$ T cells were negatively associated with learning and visuospatial skills, but did not correlate with global cognition, verbal memory, confrontation naming, verbal fluency or executive functioning (52). We also found evidence suggesting that $CD8^+$ T cells were related to cognitive functioning. After PA, the frequency of $CD8^+$ T cells in the blood decreased, which was associated with higher scores on tests of processing speed and executive function, though a movement into the CSF from the periphery was not reflected in our data set and may be limited by the small sample size. Lueg et al. reported that activated $CD4^+$ T cells in the CSF and blood were not associated with impairments in any cognitive domain in MCI patients (52). However, we found that after PA, increased $CD4^+$ T cells correlated with better executive functioning, suggesting that PA may drive relationships between the peripheral immune response and cognition not identified in prior studies.

Currently, little is known about B cells and cognition in individuals with aMCI. At baseline, we found predominantly changes in CSF-derived naïve and memory B cells in sedentary aMCI patients. Fewer naïve B cells and more memory B cells in the CSF were associated with improved attention and concentration and verbal fluency. After PA, however, cognition was only associated with changes in B cell subsets in the periphery. As peripheral naïve B cells increased and memory B cells decreased, individuals with aMCI had higher scores on measures of attention and concentration, and executive function. This recapitulates studies examining B cell subsets and global cognition in AD; naïve B cells in the blood were positively correlated with global cognition scores, while memory B cells were negatively correlated with global cognition scores (65).

Overall, our findings indicate that prior to PA in sedentary aMCI patients, correlations between B cell subsets and cognition are limited to the CSF while T cell correlations are limited to the blood. However, after PA, correlations between cognition and both B cells and T cells were found exclusively in the periphery. We also found that increased naïve B cells and $CD4^+$ T cells, and decreased memory B cells and $CD8^+$ T cells, were associated with better neurocognitive performance. This replicates our findings that identified increased naïve B cell and decreased memory B cell populations in the blood associated with less Aβ deposition in the hippocampus. Furthermore, our findings suggest that T cell subsets may be particularly associated with aspects of executive functioning. Specifically, decreased $CD4^+$ T cells and increased $CD8^+$ T cells in the CSF were associated with increased Aβ in the frontal cortex, dorsolateral prefrontal cortex, and the orbitofrontal cortex, all regions associated with executive function. Interestingly, although naïve and memory B cells were associated with changes in Aβ deposition in the hippocampus, none of the memory tasks showed significant associations between overall B cells and B cell subsets. On the other hand, T cell subsets were correlated with both Aβ burden in cortical regions associated with executive function and clinical measures of executive function, suggesting a closer association between executive functioning and T cell subsets. This highlights the need to look at long-term cognitive decline and these adaptive immune cell subsets, with additional preclinical mechanistic studies to understand the complex neuroimmune response.

Exercise interventions in MCI patients

Exercise is a lifestyle factor that can improve cognition in normal subjects, but more recently, exercise and PA have also been examined in individuals with MCI. Several systematic reviews and meta-analyses look at observational studies and randomized control trials in MCI and provide evidence suggesting an exercise benefit for incidence of MCI (6). A large

multi-national epidemiological study examined the frequency of MCI in individuals that met the weekly PA recommendation (i.e. 150 minutes of moderate-to-vigorous PA), compared to those that did not, and found that failing to meet the weekly PA recommendation was associated with increased risk of MCI (68). Similarly, another study found that any frequency of moderate exercise, as opposed to light or vigorous exercise, significantly reduced the odds of developing MCI (37). Several studies report improvements in global cognition after PA for aMCI patients (7, 9, 10, 69), including an association between physically active individuals and better scores on a global screening measure (70). Some also show evidence for improvements in attention (7), executive functioning (7, 10), verbal fluency (11), and memory (7, 9, 10). It should also be noted that some of these results do not reflect clinically meaningful changes in cognition (11), and/or inconclusive results (71).

The aforementioned studies relied on self-reported exercise data, though some used actigraphy data to categorize the frequency and intensity of exercise for aMCI patients. Healthy older individuals with low levels of daily PA (as measured by actigraphy) had more than a 2-fold risk of developing AD relative to physically active individuals (72). Another actigraph study found that increased levels of PA were associated with higher cognitive scores, but only in subjects who were cognitively normal, while one MCI investigation did not find any association between PA and improved cognitive performance (73). Falck et al. suggested this may be due to elevated rates of sedentary behavior in the MCI cohort (73) that may also influence the magnitude of effects of any PA on these patients, be it vigorous (i.e. AET) or mild (i.e. ST) interventions.

Overview of exercise-induced alterations to adaptive immunity

During and immediately after acute exercise, lymphocytes are mobilized in the blood (lymphocytosis) in an intensity-dependent manner (74, 75). Several mechanisms have been proposed to explain lymphocytosis following exercise. Exercise increases sympathetic activity, leading to greater concentrations of epinephrine and norepinephrine, which in turn stimulate lymphocyte proliferation, differentiation, and cytokine secretion (74). Increased catecholamine release acutely following exercise mobilizes adaptive immune cells through interactions with β-adrenoceptors expressed on lymphocyte surface (75). The largest changes occur in $CD8^+$ T cells, which have high β2-adrenergic receptor expression on their surface, leading to preferential mobilization (75). However, $CD4^+$ T cells and B cells also express β2-adrenergic receptors and undergo lymphocytosis after exercise, albeit to a lesser extent. Exercise preferentially mobilizes subsets of T cells, including a subset of anti-inflammatory Tregs (76-78). There are also subset-specific changes in B cells during exercise, with the greatest increase in immature B cells, followed by memory B cells, and then naïve B cells (77). These data indicate that lymphocyte subpopulations are differentially impacted during exercise and thus may play different roles in post-exercise immune function.

Within 1-2 hours post-exercise, however, there is a transient decline in circulating adaptive immune cells (lymphopenia) in the blood. Lymphopenia can be attributed to two major factors: apoptosis and lymphocyte redistribution (79). High catecholamine levels and greater oxidative stress induce lymphocyte apoptosis, though this mechanism accounts for less than 10% of lymphopenia observed after exercise (79). Lymphopenia is predominantly secondary to a redistribution of lymphocytes, particularly T cells, throughout the body. A study using cell fluorescent trafficking in mice found that $CD3^+$ T cells preferentially move from the spleen to lymphoid and non-lymphoid organs, including Peyer's patch, lungs, and bone marrow after exercise (74). Exercise also increased memory $CD8^+$ T cells expressing receptors that promote trafficking to peripheral organs, and these cells exhibit strong effector functions (80). However, outside of the aforementioned study showing a preferential increase in circulating immature B cells, little is known about B cell trafficking or apoptosis after exercise, making them a promising subject for future research. Thus, exercise may enhance immune function by promoting the redistribution of adaptive immune cells to detect antigens in remote tissues to perform secondary effector functions.

In healthy elderly adults, lifelong exercise alters baseline levels of circulating adaptive immune cells. Duggal et al. recently compared immune profiles in the blood of healthy sedentary young adults, sedentary older adults (55-79 years), and older lifelong cyclists to determine how T and B cell levels change in the blood of older individuals (81). Physically inactive older individuals had fewer T cells in their blood. However, in master cyclists, circulating T cell levels were comparable to young healthy donors. Researchers also found that sedentary older adults had more unswitched memory B cells in the blood not found in master cyclists. Together, these data suggest that age affects B and T cell profiles, but a lifetime of physical activity can preserve a "young" immune phenotype in the blood (81).

Finally, it should also be noted that an excess of anti-inflammatory regulatory immune cells can lead to immunosuppression after strenuous exercise and/or prolonged training. Marathon-trained runners had a higher percentage of interleukin (IL)-10^+ and TGF-β^+ $CD4^+$ T cells in the blood than untrained controls (82). Furthermore, high intensity exhaustive exercise results in prolonged lymphopenia, impairs T cell function, and decreases production of secretory IgA, an antibody produced by B cells (83). While this immunosuppression increases the risk of upper respiratory infections in elite athletes, the immunosuppressive effect of exercise could also counteract the systemic inflammation that naturally occurs in aging MCI patients (84, 85).

Exercise interventions and adaptive immune responses in MCI patients

Alterations to immunity may be one mechanism by which exercise interventions benefit MCI patients. Several studies examined the effect of exercise training on immunity, particularly cytokines, in MCI patients. Serum levels of the anti-inflammatory cytokine, IL-10 and the neuroprotective growth factor, brain derived growth factor (BDNF), increase after 28 weeks of strength training and 16 weeks of multimodal physical training, respectively (12, 13). As shown by Stigger et al. in a recent meta-analysis, PA also significantly decreases the

pro-inflammatory cytokines, TNF- α and IL-6 (13, 86). IL-6, which functions as both a cytokine and myokine (muscle-derived cytokine), increases immediately after a high bout of intense cycling. Multimodal physical training reduced serum levels of IL-6 and high intensity exercise training reduced IL-6 levels at rest (13, 14). Sixteen weeks of multimodal training for MCI patients found that declining levels of IL-6 and TNF-α, as well as an increased BDNF, was associated with improved cognition (13).

Only one MCI study examined changes in overall immune cell populations, with findings indicating that strength training reduced leukocyte and lymphocyte populations compared to baseline levels, but this study did not specifically examine either B or T cell subsets (12). Chupel et al. also showed that 28 weeks of strength training reduced leukocyte counts, particularly lymphocytes, relative to baseline in MCI patients, but not healthy controls (12). We did not see an effect in overall lymphocyte populations after 1-year of AET compared to ST interventions in our aMCI sample. Instead, we found that increased PA in previously sedentary individuals altered the adaptive immune responses to Aβ deposition in the brain and cognitive function, though the complexity of the responses suggest several mechanisms working in synergy during aMCI.

Study limitations

While our study provides preliminary data suggesting PA-induced immunomodulation of adaptive immune cell subsets were associated with Aβ deposition and subtle changes in cognition, only a small subset of participants in the clinical trial completed immunophenotyping, PET imaging, and cognitive testing. For example, only 5 subjects had both pre- and post-PA blood and CSF collection, so we could not analyze adaptive immune changes utilizing a within-subject design. This limited our analysis to two different groups of subjects, those at sedentary baseline and those that had post-PA blood draws and lumbar punctures. Future studies should examine immunomodulation by PA in a within-subject manner to look at how immune populations change within a particular individual to determine if baseline levels of lymphocytes impact the effect of PA on the immune system, as well as neurocognitive functioning and Aβ deposition. Because we observed no differences in the immunophenotypes in our smaller cohorts of participants, we concluded that AET and ST groups could be pooled into one larger PA cohort. We utilized this larger PA cohort when assessing the relationship between immunity and cognition, as well as immunity and Aβ deposition. We cannot exclude the possibility that by combining these cohorts, we could be obscuring the true relationship between cognition and adaptive immunity. Thus, our results only provide preliminary evidence of immune-mediated changes in neurocognitive functioning and Aβ burden after PA in patients suffering from aMCI, and may underestimate or overestimate the true effect of PA on immunity, cognition, and Aβ burden. Our conclusions should be interpreted with caution and future studies should confirm our findings in larger cohorts of aMCI patients.

CONCLUSION

Our study is unique in that it is the first to relate changes in B cell and T cell subsets to neurocognitive function and Aβ deposition in the aMCI brain after one year of PA. Preliminary data from our small cohort of aMCI patients suggest several key ways that PA may impact cognitive functioning and Aβ in aMCI. First, increased Aβ load in the hippocampus was associated with fewer naïve B cells and more memory B cells in the blood. However, there was no relationship between performance on memory tasks and B cell subsets in either the blood or CSF after PA. Instead, increased memory B cells and diminished naïve B cells were associated with lower attention and concentration scores. Second, CSF-derived $CD4^+$ T cells decreased, and $CD8^+$ T cells increased, with more Aβ deposition in brain regions associated with executive function (20). Furthermore, our data showed that aMCI patients with lower executive function scores had fewer $CD4^+$ T cells and more $CD8^+$ T cells in the blood. This suggests that PA-induced global alterations in T cell subsets may be associated with both the harmful accumulation of Aβ and associated changes in neurocognitive functioning. Finally, we found that after PA, there was an inverse relationship between memory/naïve B cells and $CD4^+/CD8^+$ T cells. Overall, increased peripheral naïve B cells and $CD4^+$ T cells are associated with better cognitive function in aMCI patients, while memory B cells and $CD8^+$ T cells are associated with greater pathology. Our findings suggest a complex interplay between physical activity, cognition, brain Aβ burden, and adaptive immunity. Larger clinical studies should aim to validate our findings and determine what mechanisms underlie PA-induced immunomodulation and its impact on clinical outcomes in MCI patients.

ACKNOWLEDGMENTS

We thank the patients who consented to participate in this study. We also thank the UT Southwestern Alzheimer's Disease Center, the NIH/NIA (P30AG12300-21), the NIH/NIAID (T32AI005284-38), the UTSWMC flow cytometry core, and the CONQUER biorepository staff for technical support. The 18^F-florbetapir PET radiotracer was provided to the study by Avid Radiopharmaceuticals.

REFERENCES

1. Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund LO, et al. Mild cognitive impairment--beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. J Intern Med. 2004;256(3):240-6.
2. Okello A, Koivunen J, Edison P, Archer HA, Turkheimer FE, Nagren K, et al. Conversion of amyloid positive and negative MCI to AD over 3 years: an 11C-PIB PET study. Neurology. 2009;73(10):754-60.
3. Waragai M, Okamura N, Furukawa K, Tashiro M, Furumoto S, Funaki Y, et al. Comparison study of amyloid PET and voxel-based morphometry analysis in mild cognitive impairment and Alzheimer's disease. J Neurol Sci. 2009;285(1-2):100-8.

4. Corey-Bloom J. Treatment trials in aging and mild cognitive impairment. Curr Top Behav Neurosci. 2012;10:347-56.
5. Strohle A, Schmidt DK, Schultz F, Fricke N, Staden T, Hellweg R, et al. Drug and Exercise Treatment of Alzheimer Disease and Mild Cognitive Impairment: A Systematic Review and Meta-Analysis of Effects on Cognition in Randomized Controlled Trials. Am J Geriatr Psychiatry. 2015;23(12):1234-49.
6. Etgen T, Sander D, Bickel H, Forstl H. Mild cognitive impairment and dementia: the importance of modifiable risk factors. Dtsch Arztebl Int. 2011;108(44):743-50.
7. Ohman H, Savikko N, Strandberg TE, Pitkala KH. Effect of physical exercise on cognitive performance in older adults with mild cognitive impairment or dementia: a systematic review. Dement Geriatr Cogn Disord. 2014;38(5-6):347-65.
8. Wang C, Yu JT, Wang HF, Tan CC, Meng XF, Tan L. Non-pharmacological interventions for patients with mild cognitive impairment: a meta-analysis of randomized controlled trials of cognition-based and exercise interventions. J Alzheimers Dis. 2014;42(2):663-78.
9. Zheng G, Xia R, Zhou W, Tao J, Chen L. Aerobic exercise ameliorates cognitive function in older adults with mild cognitive impairment: a systematic review and meta-analysis of randomised controlled trials. Br J Sports Med. 2016.
10. Cammisuli DM, Innocenti A, Franzoni F, Pruneti C. Aerobic exercise effects upon cognition in Mild Cognitive Impairment: A systematic review of randomized controlled trials. Arch Ital Biol. 2017;155(1-2):54-62.
11. Gates N, Fiatarone Singh MA, Sachdev PS, Valenzuela M. The effect of exercise training on cognitive function in older adults with mild cognitive impairment: a meta-analysis of randomized controlled trials. Am J Geriatr Psychiatry. 2013;21(11):1086-97.
12. Chupel MU, Direito F, Furtado GE, Minuzzi LG, Pedrosa FM, Colado JC, et al. Strength Training Decreases Inflammation and Increases Cognition and Physical Fitness in Older Women with Cognitive Impairment. Front Physiol. 2017;8:377.
13. Nascimento CM, Pereira JR, de Andrade LP, Garuffi M, Talib LL, Forlenza OV, et al. Physical exercise in MCI elderly promotes reduction of pro-inflammatory cytokines and improvements on cognition and BDNF peripheral levels. Curr Alzheimer Res. 2014;11(8):799-805.
14. Bengtsson Lindberg MW, Lotta; Vestberg, Susanna; Jacobsson, Helene; Wisen, Anita. Exercise-induced Release of Cytokines/Myokines in a Single Exercise Test before and after a Training Intervention in Patients with Mild Cognitive Impairment. International Jounral of Physical Therapy & Rehabilitation. 2017;3(138).
15. Monson NL, Ireland SJ, Ligocki AJ, Chen D, Rounds WH, Li M, et al. Elevated CNS inflammation in patients with preclinical Alzheimer's disease. J Cereb Blood Flow Metab. 2014;34(1):30-3.
16. Stowe AM, Ireland SJ, Ortega SB, Chen D, Huebinger RM, Tarumi T, et al. Adaptive lymphocyte profiles correlate to brain Abeta burden in patients with mild cognitive impairment. Journal of neuroinflammation. 2017;14(1):149.
17. Tarumi T, Harris TS, Hill C, German Z, Riley J, Turner M, et al. Amyloid burden and sleep blood pressure in amnestic mild cognitive impairment. Neurology. 2015;85(22):1922-9.
18. Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, et al. Current concepts in mild cognitive impairment. Archives of neurology. 2001;58(12):1985-92.
19. Fujimoto N, Prasad A, Hastings JL, Arbab-Zadeh A, Bhella PS, Shibata S, et al. Cardiovascular effects of 1 year of progressive and vigorous exercise training in previously sedentary individuals older than 65 years of age. Circulation. 2010;122(18):1797-805.
20. Yuan P, Raz N. Prefrontal cortex and executive functions in healthy adults: a meta-analysis of structural neuroimaging studies. Neurosci Biobehav Rev. 2014;42:180-92.
21. Petersen RC, Smith GE, Waring SC, Ivnik RJ, Tangalos EG, Kokmen E. Mild cognitive impairment: clinical characterization and outcome. Arch Neurol. 1999;56(3):303-8.
22. Roberts RO, Knopman DS, Mielke MM, Cha RH, Pankratz VS, Christianson TJ, et al. Higher risk of progression to dementia in mild cognitive impairment cases who revert to normal. Neurology. 2014;82(4):317-25.
23. Mitchell AJ, Shiri-Feshki M. Rate of progression of mild cognitive impairment to dementia--meta-analysis of 41 robust inception cohort studies. Acta Psychiatr Scand. 2009;119(4):252-65.
24. Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med. 2004;256(3):183-94.
25. Petersen RC, Bennett D. Mild cognitive impairment: is it Alzheimer's disease or not? J Alzheimers Dis. 2005;7(3):241-5; discussion 55-62.
26. Ferman TJ, Smith GE, Kantarci K, Boeve BF, Pankratz VS, Dickson DW, et al. Nonamnestic mild cognitive impairment progresses to dementia with Lewy bodies. Neurology. 2013;81(23):2032-8.
27. Petersen RC, Roberts RO, Knopman DS, Geda YE, Cha RH, Pankratz VS, et al. Prevalence of mild cognitive impairment is higher in men. The Mayo Clinic Study of Aging. Neurology. 2010;75(10):889-97.
28. Roberts RO, Geda YE, Knopman DS, Cha RH, Pankratz VS, Boeve BF, et al. The incidence of MCI differs by subtype and is higher in men: the Mayo Clinic Study of Aging. Neurology. 2012;78(5):342-51.
29. Green RC, Cupples LA, Go R, Benke KS, Edeki T, Griffith PA, et al. Risk of dementia among white and African American relatives of patients with Alzheimer disease. JAMA. 2002;287(3):329-36.
30. Caselli RJ, Dueck AC, Osborne D, Sabbagh MN, Connor DJ, Ahern GL, et al. Longitudinal modeling of age-related memory decline and the APOE epsilon4 effect. N Engl J Med. 2009;361(3):255-63.
31. Ng TP, Feng L, Nyunt MS, Feng L, Gao Q, Lim ML, et al. Metabolic Syndrome and the Risk of Mild Cognitive Impairment and Progression to Dementia: Follow-up of the Singapore Longitudinal Ageing Study Cohort. JAMA Neurol. 2016;73(4):456-63.
32. Vassilaki M, Aakre JA, Cha RH, Kremers WK, St Sauver JL, Mielke MM, et al. Multimorbidity and Risk of Mild Cognitive Impairment. J Am Geriatr Soc. 2015;63(9):1783-90.
33. Singh B, Mielke MM, Parsaik AK, Cha RH, Roberts RO, Scanlon PD, et al. A prospective study of chronic obstructive pulmonary disease and the risk for mild cognitive impairment. JAMA Neurol. 2014;71(5):581-8.
34. Geda YE, Roberts RO, Mielke MM, Knopman DS, Christianson TJ, Pankratz VS, et al. Baseline neuropsychiatric symptoms and the risk of incident mild cognitive impairment: a population-based study. Am J Psychiatry. 2014;171(5):572-81.

35. Roberts RO, Knopman DS, Geda YE, Cha RH, Pankratz VS, Baertlein L, et al. Association of diabetes with amnestic and nonamnestic mild cognitive impairment. Alzheimers Dement. 2014;10(1):18-26.
36. Verghese J, LeValley A, Derby C, Kuslansky G, Katz M, Hall C, et al. Leisure activities and the risk of amnestic mild cognitive impairment in the elderly. Neurology. 2006;66(6):821-7.
37. Geda YE, Roberts RO, Knopman DS, Christianson TJ, Pankratz VS, Ivnik RJ, et al. Physical exercise, aging, and mild cognitive impairment: a population-based study. Arch Neurol. 2010;67(1):80-6.
38. Mufson EJ, Binder L, Counts SE, DeKosky ST, de Toledo-Morrell L, Ginsberg SD, et al. Mild cognitive impairment: pathology and mechanisms. Acta Neuropathol. 2012;123(1):13-30.
39. Lueg G, Gross CC, Lohmann H, Johnen A, Kemmling A, Deppe M, et al. Clinical relevance of specific T-cell activation in the blood and cerebrospinal fluid of patients with mild Alzheimer's disease. Neurobiology of aging. 2015;36(1):81-9.
40. Biragyn A, Aliseychik M, Rogaev E. Potential importance of B cells in aging and aging-associated neurodegenerative diseases. Semin Immunopathol. 2017;39(3):283-94.
41. Costantini E, D'Angelo C, Reale M. The Role of Immunosenescence in Neurodegenerative Diseases. Mediators Inflamm. 2018;2018:6039171.
42. Fulop T, Larbi A, Dupuis G, Le Page A, Frost EH, Cohen AA, et al. Immunosenescence and Inflamm-Aging As Two Sides of the Same Coin: Friends or Foes? Front Immunol. 2017;8:1960.
43. Selvaraj UM, Poinsatte K, Torres V, Ortega SB, Stowe AM. Heterogeneity of B Cell Functions in Stroke-Related Risk, Prevention, Injury, and Repair. Neurotherapeutics. 2016;13(4):729-47.
44. Macaulay R, Akbar AN, Henson SM. The role of the T cell in age-related inflammation. Age (Dordr). 2013;35(3):563-72.
45. Di Benedetto S, Muller L, Wenger E, Duzel S, Pawelec G. Contribution of neuroinflammation and immunity to brain aging and the mitigating effects of physical and cognitive interventions. Neurosci Biobehav Rev. 2017;75:114-28.
46. Brosseron F, Krauthausen M, Kummer M, Heneka MT. Body fluid cytokine levels in mild cognitive impairment and Alzheimer's disease: a comparative overview. Mol Neurobiol. 2014;50(2):534-44.
47. Buchhave P, Janciauskiene S, Zetterberg H, Blennow K, Minthon L, Hansson O. Elevated plasma levels of soluble CD40 in incipient Alzheimer's disease. Neurosci Lett. 2009;450(1):56-9.
48. Contin C, Pitard V, Delmas Y, Pelletier N, Defrance T, Moreau JF, et al. Potential role of soluble CD40 in the humoral immune response impairment of uraemic patients. Immunology. 2003;110(1):131-40.
49. Gray D, Dullforce P, Jainandunsing S. Memory B cell development but not germinal center formation is impaired by in vivo blockade of CD40-CD40 ligand interaction. J Exp Med. 1994;180(1):141-55.
50. Diniz BS, Teixeira AL, Ojopi EB, Talib LL, Mendonca VA, Gattaz WF, et al. Higher serum sTNFR1 level predicts conversion from mild cognitive impairment to Alzheimer's disease. J Alzheimers Dis. 2010;22(4):1305-11.
51. Magaki S, Yellon SM, Mueller C, Kirsch WM. Immunophenotypes in the circulation of patients with mild cognitive impairment. J Psychiatr Res. 2008;42(3):240-6.
52. Lueg G, Gross CC, Lohmann H, Johnen A, Kemmling A, Deppe M, et al. Clinical relevance of specific T-cell activation in the blood and cerebrospinal fluid of patients with mild Alzheimer's disease. Neurobiol Aging. 2015;36(1):81-9.
53. Stowe AM, Ireland SJ, Ortega SB, Chen D, Huebinger RM, Tarumi T, et al. Adaptive lymphocyte profiles correlate to brain Abeta burden in patients with mild cognitive impairment. J Neuroinflammation. 2017;14(1):149.
54. Saresella M, Calabrese E, Marventano I, Piancone F, Gatti A, Calvo MG, et al. PD1 negative and PD1 positive CD4+ T regulatory cells in mild cognitive impairment and Alzheimer's disease. J Alzheimers Dis. 2010;21(3):927-38.
55. Le Page A, Garneau H, Dupuis G, Frost EH, Larbi A, Witkowski JM, et al. Differential Phenotypes of Myeloid-Derived Suppressor and T Regulatory Cells and Cytokine Levels in Amnestic Mild Cognitive Impairment Subjects Compared to Mild Alzheimer Diseased Patients. Front Immunol. 2017;8:783.
56. Scheff SW, Price DA, Ansari MA, Roberts KN, Schmitt FA, Ikonomovic MD, et al. Synaptic change in the posterior cingulate gyrus in the progression of Alzheimer's disease. J Alzheimers Dis. 2015;43(3):1073-90.
57. Small GW, Siddarth P, Kepe V, Ercoli LM, Burggren AC, Bookheimer SY, et al. Prediction of cognitive decline by positron emission tomography of brain amyloid and tau. Arch Neurol. 2012;69(2):215-22.
58. Koivunen J, Scheinin N, Virta JR, Aalto S, Vahlberg T, Nagren K, et al. Amyloid PET imaging in patients with mild cognitive impairment: a 2-year follow-up study. Neurology. 2011;76(12):1085-90.
59. Maruszak A, Thuret S. Why looking at the whole hippocampus is not enough-a critical role for anteroposterior axis, subfield and activation analyses to enhance predictive value of hippocampal changes for Alzheimer's disease diagnosis. Front Cell Neurosci. 2014;8:95.
60. Merlini M, Kirabali T, Kulic L, Nitsch RM, Ferretti MT. Extravascular CD3+ T Cells in Brains of Alzheimer Disease Patients Correlate with Tau but Not with Amyloid Pathology: An Immunohistochemical Study. Neurodegener Dis. 2018;18(1):49-56.
61. Fisher Y, Strominger I, Biton S, Nemirovsky A, Baron R, Monsonego A. Th1 polarization of T cells injected into the cerebrospinal fluid induces brain immunosurveillance. J Immunol. 2014;192(1):92-102.
62. Cao C, Arendash GW, Dickson A, Mamcarz MB, Lin X, Ethell DW. Abeta-specific Th2 cells provide cognitive and pathological benefits to Alzheimer's mice without infiltrating the CNS. Neurobiol Dis. 2009;34(1):63-70.
63. Oberstein TJ, Taha L, Spitzer P, Hellstern J, Herrmann M, Kornhuber J, et al. Imbalance of Circulating Th17 and Regulatory T Cells in Alzheimer's Disease: A Case Control Study. Front Immunol. 2018;9:1213.
64. Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, et al. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. Science. 2010;330(6012):1774.
65. Bulati M, Buffa S, Martorana A, Gervasi F, Camarda C, Azzarello DM, et al. Double negative (IgG+IgD-CD27-) B cells are increased in a cohort of moderate-severe Alzheimer's disease patients and show a pro-inflammatory trafficking receptor phenotype. J Alzheimers Dis. 2015;44(4):1241-51.

66. Simen AA, Bordner KA, Martin MP, Moy LA, Barry LC. Cognitive dysfunction with aging and the role of inflammation. Ther Adv Chronic Dis. 2011;2(3):175-95.
67. Serre-Miranda C, Roque S, Santos NC, Portugal-Nunes C, Costa P, Palha JA, et al. Effector memory CD4(+) T cells are associated with cognitive performance in a senior population. Neurol Neuroimmunol Neuroinflamm. 2015;2(1):e54.
68. Vancampfort D, Stubbs B, Lara E, Vandenbulcke M, Swinnen N, Koyanagi A. Mild cognitive impairment and physical activity in the general population: Findings from six low- and middle-income countries. Exp Gerontol. 2017;100:100-5.
69. Tan JP, Li N, Gao J, Wang LN, Zhao YM, Yu BC, et al. Optimal cutoff scores for dementia and mild cognitive impairment of the Montreal Cognitive Assessment among elderly and oldest-old Chinese population. J Alzheimers Dis. 2015;43(4):1403-12.
70. Innocenti A, Cammisuli DM, Sgromo D, Franzoni F, Fusi J, Galetta F, et al. Lifestyle, Physical Activity and Cognitive Functions: the impact on the scores of Montreal Cognitive Assessment (MoCa). Arch Ital Biol. 2017;155(1-2):25-32.
71. Song JY, Sohn JW, Jeong HW, Cheong HJ, Kim WJ, Kim MJ. An outbreak of post-acupuncture cutaneous infection due to Mycobacterium abscessus. BMC infectious diseases. 2006;6:6.
72. Buchman AS, Boyle PA, Yu L, Shah RC, Wilson RS, Bennett DA. Total daily physical activity and the risk of AD and cognitive decline in older adults. Neurology. 2012;78(17):1323-9.
73. Falck RS, Landry GJ, Best JR, Davis JC, Chiu BK, Liu-Ambrose T. Cross-Sectional Relationships of Physical Activity and Sedentary Behavior With Cognitive Function in Older Adults With Probable Mild Cognitive Impairment. Phys Ther. 2017;97(10):975-84.
74. Kruger K, Lechtermann A, Fobker M, Volker K, Mooren FC. Exercise-induced redistribution of T lymphocytes is regulated by adrenergic mechanisms. Brain Behav Immun. 2008;22(3):324-38.
75. Graff RM, Kunz HE, Agha NH, Baker FL, Laughlin M, Bigley AB, et al. beta2-Adrenergic receptor signaling mediates the preferential mobilization of differentiated subsets of CD8+ T-cells, NK-cells and non-classical monocytes in response to acute exercise in humans. Brain Behav Immun. 2018.
76. Sanders VM. The beta2-adrenergic receptor on T and B lymphocytes: do we understand it yet? Brain Behav Immun. 2012;26(2):195-200.
77. Turner JE, Spielmann G, Wadley AJ, Aldred S, Simpson RJ, Campbell JP. Exercise-induced B cell mobilisation: Preliminary evidence for an influx of immature cells into the bloodstream. Physiol Behav. 2016;164(Pt A):376-82.
78. Wilson LD, Zaldivar FP, Schwindt CD, Wang-Rodriguez J, Cooper DM. Circulating T-regulatory cells, exercise and the elite adolescent swimmer. Pediatr Exerc Sci. 2009;21(3):305-17.
79. Campbell JP, Turner JE. Debunking the Myth of Exercise-Induced Immune Suppression: Redefining the Impact of Exercise on Immunological Health Across the Lifespan. Front Immunol. 2018;9:648.
80. Campbell JP, Riddell NE, Burns VE, Turner M, van Zanten JJ, Drayson MT, et al. Acute exercise mobilises CD8+ T lymphocytes exhibiting an effector-memory phenotype. Brain Behav Immun. 2009;23(6):767-75.
81. Duggal NA, Pollock RD, Lazarus NR, Harridge S, Lord JM. Major features of immunesenescence, including reduced thymic output, are ameliorated by high levels of physical activity in adulthood. Aging Cell. 2018;17(2).
82. Rehm K, Sunesara I, Marshall GD. Increased Circulating Anti-inflammatory Cells in Marathon-trained Runners. Int J Sports Med. 2015;36(10):832-6.
83. Pedersen BK, Toft AD. Effects of exercise on lymphocytes and cytokines. Br J Sports Med. 2000;34(4):246-51.
84. Shaw DM, Merien F, Braakhuis A, Dulson D. T-cells and their cytokine production: The anti-inflammatory and immunosuppressive effects of strenuous exercise. Cytokine. 2018;104:136-42.
85. Trollor JN, Smith E, Baune BT, Kochan NA, Campbell L, Samaras K, et al. Systemic inflammation is associated with MCI and its subtypes: the Sydney Memory and Aging Study. Dement Geriatr Cogn Disord. 2010;30(6):569-78.
86. Stigger F, Marcolino MAZ, Portela KM, Plentz RDM. Effects of Exercise on Inflammatory, Oxidative and Neurotrophic Biomarkers on Cognitively Impaired Individuals Diagnosed with Dementia or Mild Cognitive Impairment: A Systematic Review and Meta-Analysis. J Gerontol A Biol Sci Med Sci. 2018.

Type 1 diabetes impairs the mobilisation of highly-differentiated CD8$^+$ T cells during a single bout of acute exercise

[1,4,5]Michelle Curran, [2]John Campbell, [1]Mark Drayson, [3]Rob Andrews, [1,6]Parth Narendran

[1] Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK.
[2] Department for Health, University of Bath, Bath, UK.
[3] University of Exeter Medical School, Exeter, UK.
[4] Drug Safety and Metabolism, Pathology, AstraZeneca, Cambridge, UK.
[5] Department of Surgery, University of Cambridge, Cambridge, UK.
[6] Department of Diabetes, The Queen Elizabeth Hospital, Birmingham, UK

ABSTRACT

Type 1 diabetes (T1D) is a T cell mediated autoimmune disease that targets and destroys insulin-secreting pancreatic beta cells. Beta cell specific T cells are highly differentiated and show evidence of previous antigen exposure. Exercise-induced mobilisation of highly-differentiated CD8$^+$ T cells facilitates immune surveillance and regulation. We aimed to explore exercise-induced T cell mobilisation in T1D. In this study, we compared the effects of a single bout of vigorous intensity exercise on T cell mobilisation in T1D and control participants. N=12 T1D (mean age 33.2yrs, predicted VO_2 max 32.2 mL/(kg·min), BMI 25.3Kg/m^2) and N=12 control (mean age 29.4yrs, predicted VO_2 max 38.5mL(kg.min), BMI 23.7Kg/m2) male participants completed a 30-minute bout of cycling at 80% predicted VO_2 max in a fasted state. Peripheral blood was collected at baseline, immediately post-exercise, and 1 hour post-exercise. Exercise-induced mobilisation was observed for T cells in both T1D and control groups. Total CD8$^+$ T cells mobilised to a similar extent in T1D (42.7%; p=0.016) and controls (39.7%; p=0.001). CD8 effector memory CD45RA$^+$ (EMRA) subset were the only T cell lineage subset to be significantly mobilised in both groups though the percentage increase of CD8$^+$ EMRA was blunted in T1D (T1D (26.5%) p=0.004, control (66.1%) p=0.010). Further phenotyping of these subsets revealed that the blunting was most evident in CD8$^+$ EMRA that expressed adhesion (CD11b: T1D 37.70%, Control 91.48%) and activation markers (CD69: T1D 29.87%, Control 161.43%), and appeared to be the most differentiated (CD27-CD28-: T1D 7.12%, Control 113.76%). CD4$^+$ T cells mobilised during vigorous intensity exercise in controls (p=0.001), but not in T1D. The blunted mobilisation response of particular T cell subsets was not due to CMV serostatus or apparent differences in exertion during the exercise bout as defined by heart rate and RPE. Predicted VO_2 max showed a trend to be lower in the T1D group than the control group but is unlikely to contribute to this blunted response. We postulate the reasons for a blunted mobilisation of differentiated CD8$^+$ EMRA cells includes differences in blood glucose, adrenaline receptor density, and sequestration of T cells in the pancreas of T1D participants. In conclusion, mobilisation of CD8$^+$ EMRA and CD4$^+$ subsets T cells is decreased in people with T1D during acute exercise.

Key words: Exercise, Physical activity, Type 1 Diabetes, Immunity, T cells

INTRODUCTION

Type 1 Diabetes (T1D) is an autoimmune disorder characterised by T cell mediated destruction of insulin secreting pancreatic beta cells. Peripheral T cells are mobilised by acute exercise in healthy individuals, and exercise may also modulate the number and function of these cells. We therefore wanted to explore the effect of exercise on T cell mobilisation in people with autoimmune T1D.

T cells in T1D

Beta cell specific T cell subsets are mainly comprised of late differentiated memory T cells that show evidence of previous antigen exposure [1-3]. Indeed, circulating islet specific CD8$^+$ T cells have been detected at similar frequencies in both T1D and non-diabetic cohorts [4]. However, antigen-experienced islet reactive CD8$^+$ T cells ($ZnT8_{186–194}$ multimer$^+$) were found sequestered in the pancreas of T1D donors, but not non-diabetic donors [4]. Islet resident CD8$^+$ T cells in the human pancreas also express CD11b, CD69, and CD103, which are markers of tissue resident memory cells [5-7]. In particular, elevated highly differentiated CD8$^+$ effector memory T cells re-expressing CD45RA (EMRA) have been found in T1D [1, 2, 8].

T cell differentiation

Towards the beginning of the linear differentiation pathway, CD8$^+$ T cells are released into the circulation as naïve T cells which are characterised by their cell surface co-expression of

Corresponding author:
Michelle Curran (michelle.curran@astrazeneca.com)
Drug Safety and Metabolism, Pathology, AstraZeneca, Cambridge, UK.

CD45RA, CCR7, CD27 and CD28 [9-14]. CD45, the leukocyte common antigen, has multiple isoforms which are differentially expressed based on maturation status [15]. Central memory (CM) cells are formed ($CD45RA^{-}CCR7^{+}$) following antigen experience and loss of CD45RA [11, 14]. Memory cells lose the expression of the high molecular weight CD45RA isoform and gain expression of the low molecular CD45RO isoform following activation [16]. Following antigen presentation, CM T cells can rapidly differentiate into T cells with effector functions [13]. CCR7 is down-regulated as CM T cells become specialised, antigen specific effector T cells. CM T cells progress to effector memory (EM) T cells through three progressively differentiated subpopulations defined based on their expression of CD27 and CD28: early differentiated (ED) ($CD27^{+}CD28^{+}$), early-like differentiated (ELD) ($CD27^{-}CD28^{+}$), and intermediate differentiated (ID) ($CD27^{+}CD28^{-}$). CD27 and CD28 diminish in a stepwise fashion following successive rounds of differentiation in response to antigen stimulus, corresponding with an increase in cytotoxic functions [17-19]. Following the loss of both CD27 and CD28, ID T cells become fully differentiated EM T cells ($CD27^{-}CD28^{-}$) [19, 20].

Re-expression of CD45RA on fully differentiated effector memory (EM) T cells (EMRA) is the final stage of T cell differentiation in the T cell lineage pathway [21] (Figure 1), which gives rise to a range of phenotypically and functionally diverse T cell subsets. This re-expression of CD45RA leads to a fixed resting memory T cell pool that can respond to recall antigens [22, 23]. Differentiation status of EMRA subsets can be identified in a number of ways; cell surface expression of markers such as CD26, CD57, and killer cell lectin-like receptor subfamily G member 1 (KLRG1) [1, 2, 8]; lack of CD27 and CD28 expression [17, 18]; and evidence of shortened telomeres indicative of successive rounds of cell division [2, 8]. Although conventionally EMRA are $CD27^{-}CD28^{-}$, there is evidence of further subdivisions of differentiation based on the differential expression of these markers [24]. Pedro Romero et al., 2007 identified small subpopulations of $CD27^{-}CD28^{+}$ and $CD27^{+}CD28^{-}$ EMRA [24]. Nonetheless, $CD27^{+}CD28^{+}$ and $CD27^{-}CD28^{-}$ EMRA make up the larger proportion of EMRA subpopulations [24, 25].

Amidst these earlier stages of differentiation exists a dynamic population $CD8^{+}$ T cells which have recently been implicated in T1D [2, 3]. Stem cell like memory T cells (T_{SCM}) ($CD27^{+}CD45RO^{-}CD95^{+}CCR7^{+}$) express naïve surface markers (CD45RA, CCR7) and are capable of extensive proliferation and self-renewal, similar to hematopoietic stem cells [26-28]. However, T_{SCM} also have the ability to rapidly acquire effector functions upon stimulation and respond to recall antigens, analogous to effector memory subsets [28, 29]. Beta cell specific T cell populations in the peripheral blood have been identified with this phenotype in T1D, indicating a role for T_{SCM} in the pathogenesis of the disease [2, 3].

In addition to progressive differentiation of $CD8^{+}$ T cell lineage subsets, $CD4^{+}$ T cells are characterised in a similar manner (Figure 1). However, in addition to this phenotypic approach, $CD4^{+}$ T cells are also defined based on their differentiation into T-helper (Th) ($CD45RO^{+}CD127^{hi}CD25^{low}$) and T-Regulatory (TRegs) ($CD4^{+}CD127^{low}CD25^{+}$) subsets [10, 30]. The T-helper subpopulations are distinguished by chemokine receptor expression and cytokine secretion patterns [10, 31-33]. These include type 1 helper (Th1) cells [10, 34, 35], type 2 helper (Th2) [10, 34], Th9 [36], Th17 [10, 37-

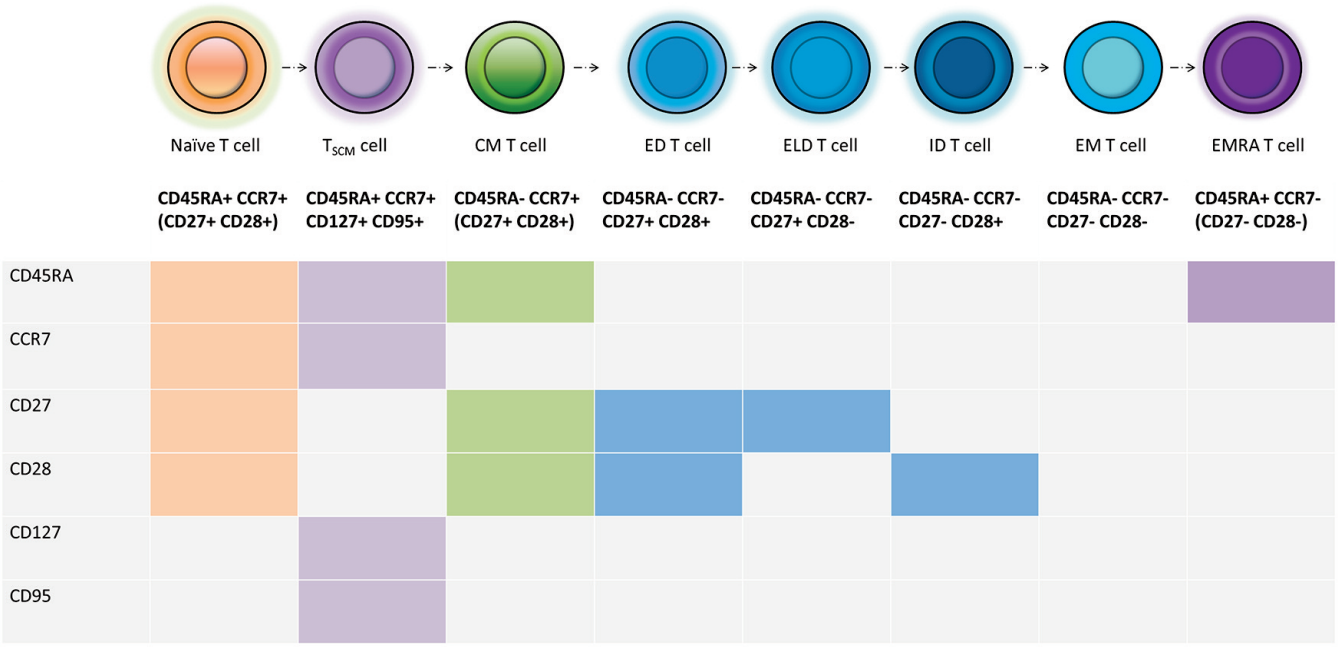


Figure 1 Linear T cell differentiation pathway
The progressive linear differentiation pathway of CD4+ and CD8+ T cell subsets; naïve T cells --> Stem cell like memory T cells (T_{SCM}) --> Central Memory (CM) --> Effector Memory (EM) --> Early Differentiated (ED) --> Early-like Differentiated (ELD) --> Intermediately differentiated (ID) --> Effector Memory Re-expressing CD45RA (EMRA). Brackets indicate additional markers that can be used for phenotypic characterisation to T cell subsets.

39], and Th22 cells [36]. $CD4^+$ TReg subpopulations consist of naïve (naTRegs) and memory TRegs (mTRegs). As described for lineage T cell subsets, naTRegs express CD45RA whereas mTRegs lose CD45RA and acquire CD45RO upon antigen exposure [11, 40]. mTRegs are an activated antigen primed subset of $CD4^+$ TRegs [41, 42]. mTREGs express TIGIT, a coinhibitory molecule [43], which supress pro-inflammatory Th1 and Th17 cells, but promote Th2 cell responses and therefore support an anti-inflammatory environment [43]. In T1D, there is strong evidence to suggest that the balance between T-helper and TRegs is disrupted. $CD4^+$ Th differentiation is skewed towards increased pro-inflammatory Th1 and Th17 cells [44, 45]. Whereas $CD4^+$ TReg function, and potentially TReg frequency, are compromised in T1D [46]. This increases immune dysregulation already upheld by memory $CD8^+$ T cell subsets.

Acute exercise and T cell mobilisation

To date, the effects of acute exercise on T cells, including those implicated in T1D pathogenesis, outlined above, have not been investigated in T1D. In healthy individuals, acute exercise induces a significant increase in the T cell frequency, proportional to exercise intensity, in the peripheral blood immediately following exercise [47].

The largest mobilisation within T cells is seen among $CD8^+$ T cells, with minimal $CD4^+$ T cell mobilisation [48-51]. Although mobilisation of $CD4^+$ T cells is minimal, there is evidence to support that exercise induces a shift from Th1 to Th2 polarization, reducing pro-inflammatory $CD4^+$ T cell phenotypes [6, 7, 79-81]. Within the $CD8^+$ T cell compartment, $CD8^+$ T cells with a highly differentiated memory phenotype are preferentially mobilised following exercise [51-56]. $CD8^+$ EMRA have been reported to increase by 450% following vigorous exercise compared to naïve $CD8^+$ T cells which increased by 84% [52]. Further studies show that $CD8^+$ effector memory (EM) and EMRA that exhibited the largest increase following exercise were fully differentiated ($CD27^-$ $CD28^-$) [53] and expressed markers of terminal differentiation and senescence (killer cell lectin-like receptor subfamily G member 1 $(KLRG1)^+CD57^+$) [54]. Whilst the cells detectable in the T1D pancreas express markers of differentiation, memory and residency [4-7], the effects of acute exercise on the mobilisation of these T cell subsets in T1D is yet to be explored.

Investigating the effects of exercise on T cell subsets in T1D may have importance for the understanding and treatment of this disease. Lymphocytosis is followed by intensity-dependent lymphopenia in the period that follows a bout of exercise [48-50, 57-59]. Following the dramatic increase in $CD8^+$ EM and EMRA frequency following exercise, it is these same subsets which exhibit the largest egress from the peripheral blood during the recovery period [53, 54]. This flux may play a role in immune regulation. Tissue redistribution of lymphocytes is one cause of exercise-induced lymphopenia [59]. Krüger et al. 2007 demonstrated using fluorescent cell tracking in mice that T cells were released from the spleen and accumulated in the lungs, bone marrow, and Peyer's patches of the mice following acute exercise [60]. Such movement of immune cells may support immune surveillance. Post-exercise lymphopenia is also thought to result partially from lymphocyte apoptosis [61-63]. Acute exercise, particularly of vigorous intensity (i.e. above 70% VO_2 max), mobilises $CD95^+$ memory T cells [61, 64]. It has been postulated that CD95 expression may indicate an apoptotic fate and subsequently create "immunological space" [65, 66]. The percentage of apoptotic lymphocytes as well as $CD95^+$ T cells increases following exhaustive exercise (80% VO_2 max) in healthy participants. [61, 63, 64]. Furthermore, mice subjected to a strenuous 90-minute treadmill running protocol showed a decrease in $CD8^+$ T cells 24 hours following exercise, and apoptosis of intestinal $CD8^+$ T cells was higher 24 hours after exercise [62]. It is hypothesised that this vacant immune space following exercise can be taken up by newly generated immature cells, creating the opportunity to reprogram immune memory. In support of this, vigorous acute exercise increases hematopoietic stem and progenitor cells (HSPC) post-exercise as well as stimulates haematopoiesis [67, 68].

Exploring the effects of acute exercise on T cells in T1D

Given that acute exercise mobilises $CD8^+$ T cells with a highly differentiated phenotype that express markers of tissue residency, and that $CD8^+$ T cells with this phenotype are thought to sustain beta cell destruction in T1D, it is important to explore the impact of acute exercise on these T cell subsets in T1D. A limited amount of research has been conducted in exercise training in T1D. Exercise training in streptozotocin-induced T1D mice significantly increased insulin content and insulin secretion compared to sedentary mice [69]. Furthermore, exercise training in non-obese diabetic (NOD) mice reduced immune cell infiltration into the pancreas and subsequently the insulitis index. This is the only exercise study in a model of T1D to demonstrate the modulatory effects of exercise on islet immunity [70]. We therefore aimed to provide a comprehensive phenotypic characterisation of exercise-induced mobilisation of potentially pathogenic T cell subsets in T1D, and compare this response to that observed in healthy participants.

METHODS

Participants

Ethical approval was granted by the Preston Research Ethics Committee (REC) for this study. Twelve controls and twelve T1D participants were recruited. All participants were male and between 16-65 years of age. Male only participants were chosen to minimise differences immune cell phenotypic and functional capacity evident in females due to higher oestrogen levels [71-73]. Participant baseline characteristics are reported in table 1. T1D participants had a clinical diagnosis of T1D, were on basal bolus insulin regime or insulin pump therapy, competent in carbohydrate content estimation of meals, were willing to test glucose through capillary testing, and were able to recognise hypoglycaemic symptoms before blood glucose fell to 3.9mmol/L. Participants did not have a history of cardiac disease or other significant illness that would prevent attendance at the study sit. All T1D participants did not have active proliferative diabetic retinopathy, autonomic neuropathy, or history of severe hypoglycaemia requiring third party assistance within the last 3 months prior to the study.

	[1]Control	[2]T1D
	mean± SD	mean± SD
Age (years)	28.8±4.6	33.2±9.7
Weight (Kg)	74.5±8.7	80.8±15.6
Height (cm)	156±55	178±7
BMI (kg/m2)	24±2	25±4
Waist circumference (cm)	86±7	90±12
Hip circumference (cm)	90±7	95±8.6
Chest Circumference (cm)	94±4	99±12
Waist-hip ratio	0.95±0.03	0.94±0.06
Body fat (%)	16.6±4.8	21.1±6.2
VO_2 Max (mL/(kg·min))	38.5±5.4	32.2±9.3
CMV index	0.42±0.47	0.51±0.5
Glucose (mmol)	5.24±0.48	8.91±3.15
Heart Rate (bpm)	68±8	74±14
Systolic BP (mmHg)	123±8	129±19
Dystolic BP (mmHg)	72±11	79±11
Number of cigarettes (per wk)[#]		
0	10	11
1-5	2	1
Alcohol intake (units per wk)[#]		
0	2	3
1-5	3	4
6-10	4	1
11-20	1	3
21-40	2	0
Job related PA (min/wk)	325±470	557±1221
Transportation PA (min/wk)	313±237	240±190
House Maintenance (min/wk)	129±122	136±129
Sport and Leisure PA (min/wk)	140±86	286±282
Light PA (min/wk)	43±45	81±91
Moderate PA (min/wk)	7.5±19	65±132
Vigorous PA (min/wk)	90±97	140±12
Time sitting (hrs/wk)	48±18	40±12
Stress score (1 year)	4±3	5±4
Stress score (1 month)	10±8	12±10
Stress score (visit 1)	7±10	6±9
Stress score (visit 2)	4±3	4±5

Table 1 Baseline Characteristics of T1D and control participants
Mean and standard deviation values for baseline characteristics in control and T1D participants.
Note: # number of participants
[1] controls n=12
[2] T1D n=12

Experimental design

Participants had one enrolment visit, where baseline demographics and anthropometric assessment was carried out (table 1). During the enrolment visit, each participant completed a non-fasted incremental sub-maximal (85% HRmax) cycle ergometer test to calculate their predicted VO_2 max. This was used to calculate workload and heart rate for the subsequent exercise visit adjusted to individual fitness [74]. The enrolment visit and exercise visit were separated by one week. Participants were asked to abstain for vigorous exercise 24 hours prior to the exercise visit. Participants were also required to record a food diary for the 24 hours prior to the exercise visit. Participants were advised to use these diaries to ensure that the same foods were consumed in the 24 hours prior to each exercise bout. The exercise visit started at 8.30am for all participants and consisted of a thirty-minute bout of cycling at 80% predicted VO_2 max. An initial fasting blood sample was taken for each participant once the cannula was inserted. The participant was then allowed to rest for a further 20 minutes before preparing for the acute exercise bout. Fasting blood samples were collected intravenously at immediately pre-exercise, immediately post-exercise, and 1 hour post-exercise. Pre- and post-exercise samples were taken whilst the participant was sitting on the cycle ergometer and sampling was strictly timed using a stopwatch. Timing for the immediately post-exercise sample was crucial because lymphocytes egress from peripheral blood within in minutes of exercise cessation [75]. All participants completed an international physical activity questionnaire (IPAQ) [76] and perceived stress questionnaires; the life scale events questionnaire [77], perceived stress scale [78], the undergraduate stress questionnaire [79], self-perceived health status [80], and the Pittsburgh sleep quality index [81].

Sample processing

All blood samples were processed under identical conditions using the same laboratory reagents and apparatus. Blood samples for immunophenotyping analysis were taken in lithium heparin vacuette tubes (95057-405, Greiner Bio-one GmbH, Frickenhausen, Germany) and placed on roller at room temperature to ensure constant mixing of the blood sample until processing. All sample processing was initiated within 2 hours of blood-draw. Haematological measures were conducted on 25µl of whole blood using an automated coulter counter (ABX Micros ES 60, HORIBA Medical). Relative cell number (cells/µl) of T cell subsets was then calculated from this.

Whole blood staining

The whole blood staining protocol was optimised prior to the start of the study. The protocol was adapted from the Clinical Immunology Service, University of Birmingham. Red blood cells were lysed by preparing whole blood in 4ml aliquots and washed with 16mls Ammonium Chloride lysis buffer (16g Ammonium Chloride (326372, Sigma-Aldrich, Dorset, UK), 2g sodium hydrogen carbonate (S/4240/60, Fisher scientific Ltd, Loughborough, UK), 0.2g EDTA (E5134, Sigma-Aldrich, Dorset, UK), and 2L ddH_2O). The sample was centrifuged at 1000g for 5 minutes. Pelleted cells were resuspended in 10mls RPMI-1640 (R0833, Sigma-Aldrich, Dorset, UK) (supplemented with 2% FBS) and centrifuged at 1000g for 5 minutes. Cells were then counted and resuspended to a concentration of 1x106 cells/ml. Cells were stained with appropriate antibodies and incubated in dark at 4°C for 20minutes. Stained cells were lysed and fixed with 500µl 1X BD FACS lysing solution (containing 14% formaldehyde) (349202, BD Biosciences, Wokingham, UK) and incubated in dark at 4°C for a further 15minutes. Fixed cells were washed (centrifuged at 1000g for 5 minutes) in 2mls phosphate-buffered saline (PBS). Pelleted cells were resuspended in 500µl PBS and stored at 4°C until flow cytometry analysis. The stability of fixed stains was assessed and confirmed that cells could be stored up to 24hours at 4°C before flow cytometry analysis. All samples were analysed using BD LSR Fortessa X-20. Parent populations (i.e. lymphocytes) were selected based on their size on FSC/SSC dot plots. Doublets were omitted by selecting the linear population shown on FSC-A/FSC-H dot plots prior to recording. Events to record

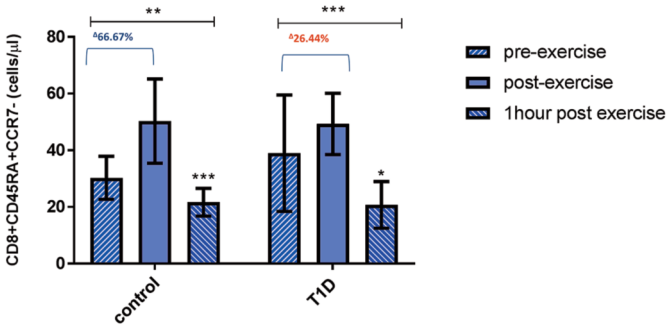


Figure 2 CD8+ EMRA during vigorous intensity exercise in T1D and control participants
Flow cytometry was used to determine the frequency (cells/µl) of CD8+ EMRA populations in control and T1D participants during vigorous intensity exercise. Error bars represent SEM.
Note:
Δ percentage change
* $p \leq 0.05$
** $p \leq 0.01$
*** $p \leq 0.001$

were set to 100,000 within the parent population gate. Compensation was carried out monthly using compensation beads and single stained cells. The most recent compensation set up was linked to each experiment. A negative control (unstained whole blood) was run for each experiment.

T cell subset analysis

Individual T cell phenotypes along the linear differentiation pathway of $CD4^+$ and $CD8^+$ T cells (Figure 1), as well as $CD4^+$ T-helper (Th) and T-Regulatory (TReg) subsets following an acute bout of vigorous intensity exercise (80% predicted VO_2 max) were measured in T1D and healthy participants. Two multi-colour flow cytometry panels were designed to phenotype lineage T cell subsets (panel 1) and CD4 T-helper/T-Regulatory subsets (panel 2) using the following anti-human monoclonal antibodies (mAbs) obtained from BD Biosciences (Wokingham, UK) (unless stated otherwise):

Panel 1 anti-CD3 PE-Cy7 (UCHT1), anti-CD4 APC-R700 (RPA-T4), anti-CD8 APC-H7 (SK1), anti-CD11b PE-CF594 (ICRF44), anti-CD27 BB515 (M-T271), anti-CD28 BV510 (CD28.2), anti-CD45RA BV786 (HI100), anti-CD69 BV650 (FN50), anti-CD95 BV421 (DX2), anti-CD127 AF647 (HIL-7R-M21), anti-CCR7 PE (3D12), anti-7-AAD PerCP-Cy5.5.
Panel 2 anti-CD3 PE-Cy7 (UCHT1), anti-CD4 APC-R700 (RPA-T4), anti-CD25 PE (M-A251), anti-CD45RO BV786 (UCHL1), anti-CD127 AF647 (HIL-7R-M21), anti-CXCR3 PE-CF594 (1C6), anti-CCR4 BV421 (1G1), anti-CCR6 BV711 (11A9), anti-IL-6R FITC (AS12), anti-TIGIT PerCP-Cy5.5 (MBSA43) (eBioscience, San Diego, CA, US), and Live/Dead-Fixable Viability Stain 780.

Data analysis

Flowjo version 10 (FlowJo LLC, Oregon) was used to analyse flow cytometry data. Doublets were removed using FSC-A versus FSH-H dot plots. Dead cells positive for 7-AAD viability stain were removed, and lymphocytes were selected based on size on SSC-A versus FSC-A dot plots. Total T cells were selected as $CD3^+$, and further selected as separate $CD4^+$ and $CD8^+$ populations. Spider gates were used within $CD4^+$ and $CD8^+$ populations to define distinct progressively differentiated subsets as outlined in supplementary Figure 1; naïve ($CD45RA^+CCR7^+$), T_{SCM} ($CD45RA^+CCR7^+CD95^+CD127^+$), central memory (CM: $CD45RA^-CCR7^+$), effector memory (EM: $CD45RA^-CCR7^-$), early differentiated (ED: $CD45RA^-CCR7^-CD27^+CD28^+$), early-like differentiated (ELD: $CD45RA^-$ $CCR7^-$ $CD27^-CD28^+$) and intermediately differentiated (ID: $CD45RA^-$ $CCR7^-CD27^+CD28^-$), and effector memory re-expressing CD45RA (EMRA: $CD45RA^+CCR7^-$). The gating strategy for T_{SCM} is displayed in supplementary Figure 2. Single cell surface expression of CD69, CD11b, CD127, and CD95 was gated on CD4 and CD8 naïve, CM, EM, and EMRA T cell subsets.

$CD4^+$ T cells were further divided into T-helper (supplementary Figure 3) and T-Regulatory (supplementary Figure 4)

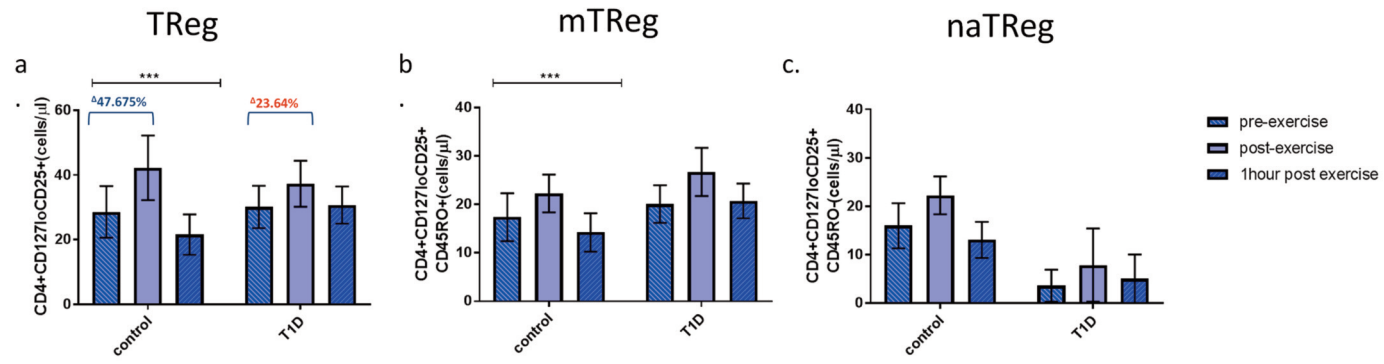


Figure 3 T-Regulatory cell subsets during vigorous intensity exercise in T1D and control participants
Flow cytometry was used to determine the frequency (cells/µl) of CD4+ TReg, mTReg, and nTReg subsets in control and T1D participants during vigorous intensity exercise. (*a*) The frequency of CD4+ TReg cells (*b*) CD4+ mTReg cells and (*c*) CD4+ naTReg cells during vigorous intensity exercise. Error bars represent SEM.
Note:
Δ percentage change
* $p \leq 0.05$
** $p \leq 0.01$
*** $p \leq 0.001$

Subset (cells/µl)	[1]Controls							[2]T1D							[d]Time (overall)	[d]Time*Group
	T1	T2	T3	[b]Time		[c]contrast	[a]Δ%	T1	T2	T3	[b]Time		[c]contrast	[a]Δ%		
CD8 naïve	100.77± 70.41	108.43± 84.76	81.54± 57.25	F(1.3, 14.5)= 0.751, p=0.436	T2	F(1, 10)= 0.368, p=0.556	7.60	48.16± 62.31	61.36± 66.97	48.68± 53.50	F(2,16)= 1.159, p=0.339	T2	F(1, 8)= 1.654, p=0.234	27.41	F (2, 34)=1.834, p=0.175	F (2, 34)=0.797, p=0.459
					T3	F(1, 10)= 2.051, p=0.180	19.08					T3	F(1, 8)= 0.156, p=0.704	1.07		
CD8 T_{SCM}	0.53±0. 69	10.65±3 4.51	0.47±1 .01	F(1.1, 9.6)= 1.036, p=0.340	T2	F(1, 9)= 1.084, p=0.325	1898. 68	9.71±2 6.74	0.88±2 .19	12.62± 36.22	F(1.02, 7.12)= 0.701, p=0.432	T2	F(1, 7)= 0.730, p=0.421	90.97	F(1.04, 16.6)= 0.052, p=0.832	F(1.04, 16.6)= 1.140, p=0.304
					T3	F(1, 9)= 0.008, p=0.931	12.62					T3	F(1, 7)= 0.081, p=0.784	30.02		
CD8 CM	27.09±1 3.66	27.70±1 6.48	25.42± 13.49	F(2, 22)= 1.343, p=0.282	T2	F(1, 11)= 0.012. p=0.914	2.25	25.82± 10.51	29.66± 17.33	17.95± 10.19	F(1.35, 4.9)= 2.209, p=0.173	T2	F(1, 8)= 2.048, p=0.190	14.90	F(1.3, 21.9)= 0.546, p=0.511	F(1.3, 21.9)= 0.255, p=0.678
					T3	F(1, 8)= 2.059, p=0.179	6.15					T3	F(1, 8)= 1.961, p=0.199	30.48		
CD8 EM	213.22± 129.35	314.69± 218.55	179.30 ±102.0 3	F(2, 22)= 1.486, p=0.248	T2	F(1, 11)= 0.06, p=0.763	47.59	157.07 ±151.5 0	269.78 ±224.4 2	124.62 ±115.2 8	F(1.2, 9.8)= 4.060, p=0.067	T2	F(1, 8)= 2.124, p=0.183	71.76	F(1.257, 21.4)= 0.822, p=0.401	F(1.257, 21.4)=0.286, p=0.651
					T3	F(1, 11)= 3.356, p=0.094	15.91					T3	F(1, 8)= 5.978, p=0.040	20.66		
CD8 ED	102.30± 97.85	123.23± 116.89	65.15± 52.83	F(2, 22)= 0.882, p=0.428	T2	F(1, 11)= 0.011, p=0.918	20.46	37.27± 64.35	53.51± 85.79	8.50±1 1.81	F(1.2, 9.3)= 3.599, p=0.085	T2	F(1, 8)= 1.289, p=0.289	43.55	F(1.2, 20.3)= 4.520, p=0.040	F(1.2, 20.3)= 0.051, p=0.864
					T3	F(1, 11)= 1.498, p=0.247	36.32					T3	F(1, 8)= 4.830, p=0.059	77.20		
CD8 ELD	38.91±3 9.00	63.49±6 2.18	33.49± 30.31	F(1.1, 12.1)= 1.390, p=0.266	T2	F(1, 11)= 0.906, p=0.362	63.17	84.05± 140.43	144.09 ±222.8 4	54.26± 81.30	F(1.2, 9.9)= 4.492, p=0.054	T2	F(1, 8)= 2.044, p=0.191	71.44	F(1.4, 24.5)= 14.969, p<0.001	F(1.4, 24.5)= 1.501, p=0.240
					T3	F(1, 11)= 2.436, p=0.147	13.94					T3	F(1, 8)= 7.006, p=0.029	35.44		
CD8 ID	25.93±2 0.99	35.35±2 6.47	19.22± 11.57	F(1.3, 13.9)= 3.252, p=0.086	T2	F(1, 11)= 1.143, p=0.308	36.29	9.18±1 2.76	14.78± 22.44	8.52±1 3.08	F(1.1, 8.75)= 2.317, p=0.163	T2	F(1, 8)= 1.819, p=0.214	60.94	F(1.4, 24)= 15.543, p<0.001	F(1.4, 24)= 0.660, p=0.476
					T3	F(1, 11)= 6.518, p=0.027	25.90					T3	F(1, 8)= 2.922, p=0.126	7.22		
CD8 EMRA	33.15±2 6.28	55.25±4 9.30	21.67± 16.27	**F(1.37, 15.1)= 7.458, p=0.010**	T2	F(1, 11)= 2.5052, p=0.180	**66.67**	39.00± 61.60	49.31± 32.36	20.75± 24.74	**F(2,16)= 8.053, p=0.004**	T2	F(1, 8)= 3.981, p=0.081	**26.45**	**F (1.5, 25)= 11.823, p=0.001**	F (1.5, 25)= 0.472, p=0.571
					T3	**F(1, 11)= 13.361, p=0.004**	34.62					T3	**F(1, 8)= 8.487, p=0.019**	46.80		

Table 2 CD8+ lineage subsets following vigorous intensity exercise

Mean, standard deviation, and statistical analysis of CD8+ sub populations for control and T1D subjects during vigorous intensity exercise. Significant results highlighted in bold changes over time were statistically significant for both groups independently i.e. p values <0.05 were considered significant, and the percentage increase was considerably blunted in the T1D (<50 Δ% compared to control highlighted in red). Note: [a] Δ% Percentage change from baseline (T1) to immediately post exercise (T2) or 1 hour post exercise (T3). [b] Results were analysed using multiple regression analysis in control and T1D groups independently. [c] Contrast displays changes from baseline (T1) to immediately post exercise (T2) and 1 hour post exercise (T3).[d] Results were analysed using multiple regression analysis in control and T1D groups combined.[1]Controls n=11, [2]T1D n=9

Subset (cells/µl)	[1]Controls							[2]T1D							[d]Time (overall)	[d]Time*Group
	T1	T2	T3	[b]Time	[c]contrast		[a]Δ%	T1	T2	T3	[b]Time	[c]contrast		[a]Δ%		
CD8 Na CD69+	2.78±2.58	5.00±5.82	3.52±3.41	F(1.2, 12.1)= 2.761, p=0.118	T2	F(1, 10)= 6.517, p=0.029	79.90	1.34±1.06	1.93±1.44	1.18±1.26	F(2,16)= 6.162, p=0.010	T2	F(1, 8)= 8.294, p=0.021	43.69	F(1.26, 22.8)= 0.907p=0.374	F(1.26, 22.8)=0.370, p=0.598
					T3	F(1, 10)= 4.602, p=0.058	26.61					T3	F(1, 8)= 1.414, p=0.269	12.12		
CD8 CM CD69+	1.04±1.33	1.92±2.17	1.32±.41	F(1.33, 13.25= 4.884, p=0.037	T2	F(1, 10)= 10.016, p=0.010	85.57	0.53±0.59	0.85±1.11	0.38±0.40	F(1.2, 9.7)= 7.190, p=0.020	T2	F(1, 8)= 8.133, p=0.021	59.98	F(1.11, 20)= 2.978, p=0.362	F(1.11, 20)= 1.905, p=0.163.
					T3	F(1, 10)= 1.160, p=0.307	27.61					T3	F(1, 8)= 3.895, p=0.084	28.86		
CD8 EM CD69+	7.91±5.60	21.44±25.80	9.28±8.97	F(2, 20)= 2.070, p=0.152	T2	F(1, 10)= 3.141, p=0.107	170.99	14.15±18.34	28.62±42.23	19.90±43.81	F(2,16)= 5.970, p=0.012	T2	F(1, 8)= 5.052, p=0.055	102.26	F (2, 34)= 1.046 p=0.362	F (2, 34)= 0.527, p=0.595
					T3	F(1, 10)= 0.095, p=0.764	17.25					T3	F(1, 8)= 2.708, p=0.138	40.62		
CD8 EMRA CD69+	1.62±.25	4.24±.34	1.96±.70	**F(2, 20)= 8.049, p=0.003**	T2	**F(1, 10)= 10.152, p=0.010**	**161.43**	4.86±7.55	6.31±5.23	2.96±4.56	**F(2,16)= 9.665, p=0.002**	T2	F(1, 8)= 4.554, p=0.065	**29.87**	F(1.2, 21.2)= 1.884 p=0.184	F(1.2, 21.2)= 1.638, p=0.217
					T3	F(1, 10)= 0.087, p=0.774	21.13					T3	F(1, 8)= 3.616, p=0.094	39.21		
CD8 Na CD11b+	2.90±2.31	4.74±6.16	3.20±2.72	F(2, 22)= 0.418, p=0.663	T2	F(1, 11)= 0.977, p=0.344	63.15	1.48±2.39	1.56±1.73	1.15±1.48	F(2,16)= 1.274, p=0.307	T2	F(1, 8)= 1.196, p=0.306	5.80	F(1.1,18.7)= 0.086 p=0.797	F(1.1,18.7)= 1.378, p=0.259
					T3	F(1, 11)= 0.067, p=0.801	10.30					T3	F(1, 8)= 0.285, p=0.608	22.46		
CD8 CM CD11b+	1.10±1.32	1.52±1.42	0.96±0.99	F(2, 22)= 2.455, p=0.109	T2	F(1, 11)= 2.497, p=0.114	37.25	0.61±0.60	0.62±0.67	0.50±0.67	F(2,16)= 1.225, p=0.313	T2	F(1, 8)= 0.013, p=0.911	1.09	F(1.22, 20.7)= 1.404, p=0.252	F(1.22, 20.7)= 1.055, p=0.319
					T3	F(1, 11)= 0.111, p=0.745	12.81					T3	F(1, 8)= 1.825, p=0.214	18.59		
CD8 EM CD11b+	14.55±19.17	32.14±46.56	14.35±16.81	F(2, 22)= 1.186, p=0.324	T2	F(1, 11)= 3.799, p=0.077	120.96	37.91±77.40	58.88±120.62	13.86±18.71	F(2,16)= 1.858, p=0.188	T2	F(1, 8)= 2.295, p=0.168	55.33	F(1.1, 18.6)= 2.040, p=0.169	F(1.1, 18.6)= 0.742, p=0.412
					T3	F(1, 11)= 0.168, p=0.689	1.37					T3	F(1, 8)= 0.692, p=0.430	63.44		
CD8 EMRA CD11b+	1.72±1.17	3.30±3.05	1.29±1.39	**F(2, 22)= 10.424, p=0.001**	T2	**F(1, 11)= 9.678, p=0.010**	**91.48**	22.09±56.35	13.76±20.34	7.86±18.66	**F(1, 16)= 6.807, p=0.007**	T2	F(1, 8)= 2.296, p=0.168	**37.70**	F(1.34, 22.8)= 1.483 p=0.243	F(1.34, 22.8)=1.428, p=0.254
					T3	F(1, 11)= 3.828, p=0.076	24.82					T3	**F(1, 8)= 14.156, p=0.006**	64.43		
CD8 Na CD127+	67.94±54.84	84.76±66.64	58.84±43.04	F(1.4, 11.17)= 0.498, p=0.530	T2	F(1, 10)= 0.076, p=0.788	24.76	46.26±66.22	58.96±70.71	46.59±56.68	F(2,16)= 0.895, p=0.428	T2	F(1, 8)= 1.642, p=0.236	27.45	F(1.24, 19.8)= 0.162 p=0.744	F(1.24, 19.8)= 0.832, p=0.397.
					T3	F(1, 10)= 1.140, p=0.311	13.39					T3	F(1, 8)= 0.012, p=0.915	0.70		
CD8 CM CD127+	21.24±13.95	23.70±14.20	20.85±10.42	F(2, 20)= 0.288, p=0.753	T2	F(1, 10)= 0.256, p=0.624	11.61	12.04±9.30	19.84±15.56	10.64±10.10	F(1.1, 8.78)= 1.437, p=0.267	T2	F(1, 8)= 1.292, p=0.289	64.86	F (2, 36)= 3.583, p=0.038	F (2, 36)= 0.810, p=0.453.
					T3	F(1, 10)= 0.468, p=0.510	1.83					T3	F(1, 8)= 1.330, p=0.282	11.62		
CD8 EM CD127+	152.49±129.64	223.97±166.96	136.06±90.49	F(1.2, 12.1)= 0.470, p=0.632	T2	F(1, 10)= 0.094, p=0.766	46.87	120.65±114.79	195.35±168.84	97.52±97.81	F(1.1, 9.1)= 2.603, p=0.105	T2	F(1, 8)= 1.068, p=0.332	61.91	F(1.2, 19.9)= 1.274 p=0.035	F(1.2, 19.9)= 0.855, p=0.385.
					T3	F(1, 10)= 1.319, p=0.277	10.78					T3	F(1, 8)= 6.288, p=0.037	19.17		
CD8 EMRA CD127+	13.15±12.71	17.90±16.17	11.41±9.04	F(1.3, 12.5)= 1.793, p=0.192	T2	F(1, 10)= 1.541, p=0.243	36.16	10.00±7.50	16.15±11.51	8.70±10.00	F(1.2, 9.7)= 3.852, p=0.074	T2	F(1, 8)= 2.333, p=0.165	61.49	F(1.24, 21)= 4.606 p=0.037	F(1.24, 21)=0.200, p=0.711
					T3	F(1, 10)= 1.398, p=0.264	13.24					T3	F(1, 8)= 5.058, p=0.055	12.98		

CD8 Na CD95+	1.06±1.20	11.50±36.36	0.71±1.45	F(2, 22)= 0.218, p=0.806	T2	F(1, 11)= 0.460, p=0.512	983.77	11.97±28.45	1.69±2.88	14.88±38.89	F(1.5, 7.3)= 0.642, p=0.454	T2	F(1, 7)= 0.201, p=0.667	85.84	F(1, 16.5)= 0.009, p=0.991	F(1, 16.5)=1.588, p=0.220
					T3	F(1, 11)= 0.193, p=0.669	33.41					T3	F(1, 7)= 0.536, p=0.488	24.35		
CD8 CM CD95+	1.42±1.17	3.94±9.92	1.54±1.98	F(2, 22)= 0.196, p=0.823	T2	F(1, 11)= 0.434, p=0.523	176.70	2.68±3.26	2.33±.93	2.35±3.38	F(1.14, 9.1)= 0.056, p=0.847	T2	F(1, 8)= 0.004, p=0.950	12.77	F(1.5, 25.2)= 2.081, p=0.155	F(1.5, 25.2)= 0.585 p=0.515
					T3	F(1, 11)= 0.117, p=0.739	8.09					T3	F(1, 8)= 0.704, p=0.426	12.23		
CD8 EM CD95+	11.53±10.99	58.31±170.75	8.27±9.83	F(2, 22)= 0.104, p=0.901	T2	F(1, 11)= 0.006, p=0.941	405.65	20.42±24.03	23.64±44.90	16.44±18.71	F(1.1, 8.7)= 0.103, p=0.777	T2	F(1, 8)= 0.051, p=0.827	15.79	F(1.4, 23)= 2.993, p=0.087	F(1.4, 23)= 0.383, p=0.605
					T3	F(1, 11)= 0.258, p=0.622	28.27					T3	F(1, 8)= 2.424, p=0.158	19.50		
CD8 EMRA CD95+	0.91±0.98	3.91±9.85	0.44±0.74	F(1.35, 14.8)= 0.909, p=0.386	T2	F(1, 11)= 1.003, p=0.338	330.92	19.02±50.96	7.65±17.02	6.85±15.61	F(1.4, 11.143)= 0.289, p=0.753	T2	F(1, 8)= 0.292, p=0.604	59.76	F(1.24, 21)= 10.025, p=0.003	F(1.24, 21)= 1.106, p=0.320
					T3	F(1, 11)= 0.380, p=0.550	51.84					T3	F(1, 8)= 1.016, p=0.343	63.96		
CD8 EMRA CD28-CD27-	12.98±13.87	27.76±30.42	9.24±7.78	**F(2, 20)= 3.912, p=0.037**	T2	F(1, 10)= 3.426, p=0.094	**113.76**	31.36±67.78	29.13±29.74	11.94±22.78	**F(1.13, 6.8)= 5.464, p=0.050**	T2	F(1, 6)= 2.061, p=0.201	**7.12**	**F(1.4, 20.5)= 8.955, p=0.004**	F(1.4, 20.5)= 1.967, p=0.174
					T3	F(1, 10)= 1.074, p=0.324	28.82					T3	**F(1, 6)= 19.107, p=0.005**	61.91		
CD8 EMRA CD28-CD27+	6.94±8.34	9.46±12.59	5.70±7.68	F(1.3, 13.1)= 3.765, p=0.066	T2	F(1, 10)= 3.041, p=0.112	36.30	3.16±4.73	4.34±5.09	2.52±3.73	F(2,12)= 1.174, p=0.342	T2	F(1, 6)= 0.829, p=0.398	37.12	F(1.35, 18.9)= 2.559, p=0.119.	F(1.35, 18.9)= 0.808, p=0.456
					T3	F(1, 10)= 2.860, p=0.122	17.94					T3	F(1, 6)= 1.295, p=0.298	20.45		
CD8 EMRA CD28+CD27-	5.81±8.82	10.34±19.88	4.43±8.52	F(2, 20)= 3.214, p=0.062	T2	F(1, 10)= 1.430, p=0.259	78.06	7.68±9.94	12.83±17.45	3.32±4.43	F(2,12)= 5.293, p=0.022	T2	F(1, 6)= 1.951, p=0.212	67.07	F(1.4, 23)= 0.409, p=0.590.	F(1.4, 23)= 1.092, p=0.328
					T3	F(1, 10)= 2.133, p=0.175	23.74					T3	F(1, 6)= 6.245, p=0.047	56.78		
CD8 EMRA CD28+CD27+	6.55±7.64	6.41±7.14	3.84±3.74	F(1.1, 10.1)= 0.114, p=0.757	T2	F(1, 10)= 0.009, p=0.926	2.26	4.41±8.14	7.25±13.92	6.01±12.95	F(2,12)= 1.185, p=0.339	T2	F(1, 6)= 1.989, p=0.208	64.38	F(1.3, 22.7)= 1.132, p=0.314	F(1.3, 22.7)= 0.083, p=0.831
					T3	F(1, 10)= 0.097, p=0.761	41.33					T3	F(1, 6)= 0.339, p=0.582	36.47		

Table 3 CD8⁺ lineage subset surface markers following vigorous intensity exercise

Mean, standard deviation, and statistical analysis of CD69+, CD11b+, CD127+, and CD95+ on CD8+ naïve, CM, EM, and EMRA populations for control and T1D participants following vigorous intensity exercise. Significant results highlighted in bold where changes over time were statistically significant for both groups independently i.e. p values <0.05 were considered significant, and the percentage increase was considerably blunted in the T1D (<50 Δ% compared to control highlighted in red)

Note:

[a] Δ% Percentage change from baseline (T1) to immediately post exercise (T2) or 1 hour post exercise (T3).

[b] Results were analysed using multiple regression analysis in control and T1D groups independently.

[c] Contrast displays changes from baseline (T1) to immediately post exercise (T2) and 1 hour post exercise (T3).

[d] Results were analysed using multiple regression analysis in control and T1D groups combined.

[1] Controls n=11,

[2] T1D n =9

subsets; $CD4^+$ T-Regulatory ($CD127^{lo}CD25^{hi}$), naïve T-Reg ($CD45RO^-CD127^{lo}CD25^{hi}$), memory T-Reg ($CD45RO^-CD127^{lo}CD25^{hi}$), T-helper (Th: $CD127^+CD25^-CD45RO^+$), Th1 ($CXCR3^+CCR6^+CCR4^-$), Th2 ($CXCR3^-CCR6^-CCR4^+$), and Th17 ($CXCR3^-CCR6^+CCR4^+$).

Statistical analysis

Statistical analysis was performed using SPSS version 24 (IBM, Chicago) and GraphPad Prism version 7 (GraphPad Software, California). Firstly, normality tests were performed on all data using Q-Q plots in SPSS. Data which was not normally distributed was logged and normality tests were repeated, confirming all subsets have normal distribution. Multiple regression analysis was used to analyse within subject's effect (time) and between subject's effects over time (time*group). Main effects of exercise are described as changes over time. Changes immediately post-exercise and 1 hours post-exercise are compared to baseline values and are reported in tables for each group under the heading "contrast". P values were reported as sphericity assumed however where mauchly's test of sphericity was violated i.e. $p \leq 0.05$, Greenhouse-Geisser corrected value was used. Student T-tests were performed on baseline characteristics. The p values, F values, and degrees of freedom (df) are reported in tables as [F = (df, df error) value, p-value]. Variation in n numbers is a result of a participant having no data for 1 hour post-exercise time-point. Data are presented in tables as mean ± standard deviation (SD) unless otherwise stated. P values ≤0.05 were considered significant. Significantly mobilised subsets in control and T1D groups, however demonstrate a blunted egress immediately post exercise in T1D group, are highlighted in the results tables in bold.

RESULTS

Participants anthropometric and physiological characteristics are shown in table 1. No statistically significant differences between groups were found for anthropometric and physiologic characteristics. The results obtained for $CD8^+$ lineage subpopulations are summarized in tables 2-3. $CD4^+$ T-Regulatory and T-helper subpopulations are summarized in table 4. Extensive T cell subpopulations analysis is included in supplementary tables 1-3.

The mobilisation $CD8^+$ EMRA T cells is blunted in T1D during vigorous intensity exercise

Lymphocytosis and $CD8^+$ T cell mobilisation occurs in both T1D and control groups as summarized in supplementary table 1. However, the most dramatic changes were observed within the $CD8^+$ lineage subsets (depicted in Figure 1) during vigorous intensity exercise in T1D and control participants. The results for $CD8^+$ lineage subsets are displayed in table 2. As anticipated, within the $CD8^+$ T cell populations, less differentiated cells such as naïve, T_{SCM}, and CM subsets did not significantly change with exercise in either group. As expected, significant changes were however observed in the more differentiated subsets such as $CD8^+$ ED, ELD, ID, EM, and EMRA subsets during vigorous exercise. Therefore, the increase in $CD8^+$ T cells is driven by mobilisation of later differentiated $CD8^+$ T cell subsets, and these changes were most significant in the $CD8^+$ EMRA subsets for each group.

Although statistically significant changes in $CD8^+$ T_{SCM} were not observed during vigorous intensity exercise in either group, increases in the number $CD8^+$ T_{SCM} immediately post-exercise were suggested in both control and T1D participants (table 3). However, during vigorous intensity exercise a blunted response is noted in the T1D group. $CD8^+$ T_{SCM} decreased following vigorous exercise by 90.97% in the T1D group but increased dramatically by 1898.69% in the control group. It is worth noting that the total cell numbers for $CD8^+$ T_{SCM} subsets were very small.

Within the later differentiated subsets, $CD8^+$ ED significantly changed during vigorous intensity exercise overall only ($p=0.040$), but this was not seen in either T1D or control groups independently. There was an overall change in $CD8^+$ ELD during vigorous intensity exercise ($p<0.001$), with a non-significant trend to change in the T1D group ($p=0.054$) but not in the control group. This change was driven by an increase in $CD8^+$ ELD post-exercise followed by a significant decrease below baseline in $CD8^+$ ELD 1 hour post vigorous exercise in the T1D group only ($p=0.029$). $CD8^+$ ID significantly changed over time with vigorous exercise ($p<0.001$) but not in either T1D or control groups independently. $CD8^+$ ID significantly decreased below baseline 1 hour post vigorous exercise in the control group only ($p=0.027$). $CD8^+$ EM showed a trend to mobilise during vigorous exercise ($p=0.067$), driven by a trend to increase post-exercise, with a decrease below baseline at 1 hour post-exercise ($p=0.040$) in the T1D group. No significant changes were observed for $CD8^+$ EM in the control group.

Finally, $CD8^+$ EMRA cell frequency significantly changed overall during vigorous intensity exercise ($p=0.001$). $CD8^+$ EMRA were significantly mobilised by vigorous intensity exercise, driven by a trend to increase post-exercise, in T1D ($p=0.004$) and control groups ($p=0.010$). There was a significant decrease 1 hour post-exercise in both T1D ($p=0.019$) and control groups ($p=0.004$). In summary, $CD8^+$ EMRA were the only $CD8^+$ T cells subset mobilised by vigorous intensity exercise in both the T1D and control group. However, the percentage increase post vigorous intensity exercise is much lower in the T1D group (control: 66.67%, T1D: 26.45%) suggesting a blunted egress of $CD8^+$ EMRA during exercise in T1D (Figure 2); however, no significant time*group differences were observed.

Vigorous intensity exercise mobilises fully differentiated $CD8^+$ EMRA T cells expressing markers of activation and adhesion in T1D and control participants, with evidence of a blunted response in T1D

A number of cell surface markers were measured on $CD8^+$ T cells to define the homing propensity and function of circulating $CD8^+$ populations in T1D and control participants. These were examined on naïve, CM, EM, and EMRA $CD8^+$ T cell subsets. These markers included CD69 (a marker of activation and tissue-resident populations), CD11b (an adhesion marker involved in lymphocyte migration), CD127 (IL-7Rα, necessary for memory $CD8^+$ T cell maintenance), and CD95 (expressed on memory subsets and a marker of apoptosis). The combination of CD27 and CD28 expression was used to define the differentiation status of $CD8^+$ EMRA subsets. Con-

Subset (cells/µl)	[1]Controls							[2]T1D							[d]Time (overall)	[d]Time*Group
	T1	T2	T3	[b]Time	[c]contrast		[a]Δ%	T1	T2	T3	[b]Time	[c]contrast		[a]Δ%		
Th1 (CXCR3+CCR6+CCR4-)	80.36±71.03	103.28±66.30	62.93±51.74	F(2, 12)= 3.533, p=0.062	T2	F(1, 6)= 1.122, p=0.330	28.51	47.51± 54.36	65.08± 56.56	58.42±61.04	F(2, 12)= 1.232, p=0.326	T2	F(1, 6)= 1.485 p=0.269	36.97	F(2, 24)= 3.865, p=0.035	F(2, 24)= 1.482, p=0.246
					T3	F(1, 6)= 4.053, p=0.091	21.69					T3	F(1, 6)= 1.027, p=0.350	22.95		
Th2 (CXCR3-CCR6-CCR4+)	27.54±45.57	34.90±46.26	19.23±28.38	F(2, 12)= 4.192, p=0.042	T2	F(1, 6)= 6.964, p=0.039	26.72	18.19± 14.33	25.20± 14.77	18.96±12.83	F(1.1, 6.7)= 0.836, p=0.406	T2	F(1, 6)= 0.467, p=0.520	38.52	F(2, 24)= 3.634, p=0.042	F(2, 24)= 0.156, p=0.857
					T3	F(1, 6)= 0.575, p=0.477	30.18					T3	F(1, 6)= 1.341, p=0.291	4.21		
Th17 (CXCR3-CCR6+CCR4+)	19.44±18.61	25.02±14.28	13.89±12.85	F(2, 12)= 3.075, p=0.084	T2	F(1, 6)= 3.561, p=0.108	28.72	11.63± 9.66	21.71± 18.75	10.75±7.80	F(1.1, 6.8)= 3.475, p=0.104	T2	F(1, 6)= 1.916, p=0.216	86.68	F(2, 24)= 6.363, p=0.006	F(2, 24)= 0.077, p=0.926
					T3	F(1, 6)= 0.548, p=0.487	28.55					T3	F(1, 6)= 8.154, p=0.029	7.58		
TReg (CD127loCD25hi)	28.60±25.24	42.23±30.07	21.56±18.79	**F(2, 16)= 6.376, p=0.009**	T2	F(1, 8)= 2.324, p=0.166	**47.65**	30.13± 17.33	37.28± 18.85	30.75±14.09	F(1.1, 6.5)= 0.948, p=0.374	T2	F(1, 6)= 0.418, p=0.542	**23.74**	**F(2, 28)= 5.401, p=0.010**	F(2, 28)= 0.519, p=0.600
					T3	F(1, 8)= 3.658, p=0.092	24.60					T3	F(1, 6)= 4.203, p=0.086	2.05		
naïve TReg (CD45RO-)	12.59±12.30	19.98±22.39	7.72±8.25	F(1.1, 9.6)= 2.182, p=0.172	T2	F(1, 9)= 1.692, p=0.226	58.69	10.08± 8.99	10.59± .63	10.03±7.60	F(2, 12)= 0.382, p=0.690	T2	F(1, 6)= 0.124, p=0.737	5.06	F(1.1, 16.1)= 1.631, p=0.221	F(1.1, 16.1)= 1.299, p=0.275
					T3	F(1, 9)= 2.712, p=0.134	38.68					T3	F(1, 6)= 4.382, p=0.081	0.48		
memory TReg(CD45RO+)	16.01±14.72	22.25±11.69	13.07±11.16	F(2, 16)= 7.222, p=0.006	T2	F(1, 8)= 2.785, p=0.134	38.98	20.05± 10.27	26.70± 13.15	20.72±8.76	F(1.1, 6.6)= 1.606, p=0.252	T2	F(1, 6)= 1.232, p=0.310	33.12	F(2, 28)= 6.632, p=0.004	F(2, 28)= 0.291, p=0.750
					T3	F(1, 8)= 4.198, p=0.075	18.34					T3	F(1, 6)= 1.8974, p=0.220	3.30		
memory TReg TIGIT+	15.04±13.39	18.49±10.48	10.63±10.30	F(2, 8)= 7.107, p=0.017	T2	F(1, 4)= 0.082, p=0.788	22.96	15.22± 7.69	20.15± 9.51	15.67±6.76	F(1.9, 6.7)= 1.641, p=0.247	T2	F(1, 6)= 1.280, p=0.301	32.37	F(1.3, 13.3)= 5.340, p=0.030	F(1.3, 13.3)= 1.069, p=0.342
					T3	F(1, 4)= 10.520, p=0.032	29.32					T3	F(1, 6)= 1.605p=0.252	2.92		

Table 4 CD4+ T-helper and T-Regulatory subsets following vigorous intensity exercise

Mean, standard deviation, and statistical analysis of CD4+ T-helper and T-Regulatory populations for control and T1D participants following vigorous intensity exercise. Significant results highlighted in bold where changes over time were statistically significant in at least one group independently i.e. p values <0.05 were considered significant.

Note: [a] Δ% Percentage change from baseline (T1) to immediately post exercise (T2). [b] Results were analysed using multiple regression analysis in control and T1D groups independently.

[c] Contrast displays changes from baseline (T1) to immediately post exercise (T2) and 1 hour post exercise (T3). [d] Results were analysed using multiple regression analysis in control and T1D groups combined.

[1] controls n=8, 2 T1D n=9

ventionally EMRA are defined as CD27-CD28-. However, more recently further subdivisions of EMRA differentiation based on the differential expression of CD27 and CD28 have been described and also shown in this study herein [24, 25].

As described earlier, $CD8^+$ EMRA are the only subsets which are significantly mobilised in both T1D and control participants. Interestingly, the percentage increase of $CD8^+$ EMRA T cells is blunted in T1D. Further phenotyping of $CD8^+$ EMRA T cell subsets using the surface markers described above show that $CD8^+$ EMRA expressing markers of activation and tissue residency (CD69), homing propensity (CD11b), and are the most differentiated ($CD27^-CD28^-$) are significantly mobilised by acute vigorous intensity exercise in both groups. Again, the percentage increase of these $CD8^+$ EMRA T cell subsets is blunted in T1D compared to control participants. No significant mobilisation was observed for $CD8^+$ EMRA expressing CD127 or CD95 in either group independently. The results for the above cell surface markers on $CD8^+$ lineage subsets during vigorous intensity exercise in T1D and control participants are displayed in table 3 and described below.

Vigorous intensity exercise significantly mobilised all but naïve subsets expressing CD69. The most profound effect was observed $CD8^+$ EMRA T cells expressing CD69. Vigorous intensity exercise resulted in the mobilisation of $CD8^+CD69^+$ EMRA T cells in T1D (p=0.002) and control groups (p=0.003), with a significant increase immediately post-exercise observed in the control group only (p=0.010). The percentage increase was blunted in the T1D group following vigorous (T1D: 29.87%, Control: 161.43%) intensity exercise (table 3).

Likewise, the most profound effect on $CD8^+$ T cell subsets expressing CD11b was observed for the EMRA. Vigorous intensity exercise significantly mobilised $CD8^+$ EMRA T cells expressing CD11b in T1D (p=0.007) and control groups (p=0.001), with no significant changes in $CD8^+CD11b^+$ naïve, CM, or EM subsets during vigorous exercise in either group. There was a significant increase immediately post-exercise in the control group only (p=0.010). However, in the T1D group, this subset significantly decreased below baseline 1 hour post vigorous exercise (p=0.006). The percentage increase of $CD8^+CD11b^+$ EMRA was blunted in the T1D group following vigorous intensity exercise (T1D: 37.70%, Control: 91.48%) (table 3).

Lastly, fully differentiated $CD8^+$ EMRA ($CD27^-CD28^-$) were the only subset of EMRA to significantly mobilise both the T1D (p=0.050) and control group (p=0.037) independently. The percentage increase post vigorous exercise was considerably blunted in the T1D group (T1D: -7.02%, control: 113.76%). There was a significant decrease below baseline 1 hour post vigorous intensity exercise in the T1D group only (p=0.005).

Vigorous intensity exercise mobilises $CD4^+$ T-Regulatory cells, mainly comprised of memory T-Regulatory cells, in control but not T1D participants

Vigorous intensity exercise mobilises $CD4^+$ T cells in control but not T1D participants (supplementary table 1). A similar pattern was observed for $CD4^+$ lineage subsets (supplementary table 2-3). Significant mobilisation patterns were also observed within the $CD4^+$ T-Regulatory and T-helper subsets, and is described below.

Total $CD4^+$ T-Regulatory cells ($CD127^{lo}CD25^{hi}$), and the naïve ($CD45RO^-CD127^{lo}CD25^{hi}$) and memory ($CD45RO^-CD127^{lo}CD25^{hi}$) compartment, were measured during vigorous intensity exercise in T1D and control participants. The results are displayed in table 4. $CD4^+$ TReg subsets were significantly mobilised by vigorous intensity exercise overall (p=0.010) and in control (p=0.009) but not T1D participants. There was a significant decrease below baseline 1 hour post-exercise overall (p=0.038), but this was not seen in either T1D or control groups independently (Figure 3a). The percentage change following exercise was much lower in the T1D compared to the control group (T1D: 23.74%, Control: 47.65%). Further delineation of $CD4^+$ TRegs revealed that memory TReg subsets were significantly mobilised by vigorous intensity exercise overall (p=0.004) and in the control group (p=0.006) (Figure 3b). There was a significant decrease below baseline overall 1 hour post-exercise (p=0.042), but this was not seen in either T1D or control group independently. Naïve TReg subsets did not significantly mobilise (Figure 3c).

Th1 ($CXCR3^+CCR6^+CCR4^-$), Th2 ($CXCR3^-CCR6^-CCR4^+$), and Th17 ($CXCR3^-CCR6^+CCR4^+$) cells were measured during vigorous intensity exercise in T1D and control participants. The results are displayed in table 4. Vigorous intensity exercise mobilised $CD4^+$ Th2 cells in control but not T1D participants. However, neither $CD4^+$ Th1 or Th17 cells significantly mobilised in either control or T1D groups independently. In summary, $CD4^+$ T-helper subsets do not significantly mobilise in T1D participants. However, some mobilisation of Th2 subsets were observed in control participants, shifting the Th1/Th2 ratio towards anti-inflammatory subsets. Th2 subsets significantly mobilised during vigorous intensity exercise in control (p=0.042) but not T1D participants, with a significant increase post-exercise in the control group (p=0.039).

DISCUSSION

This study has, for the first time, characterised the effects of acute exercise on the mobilisation of T cell subsets in people with T1D. The use of surface markers to define T cell function and fate improves understanding of the specific subpopulations mobilised during exercise in a T cell mediated autoimmune disease.

Acute exercise causes an intensity-dependent lymphocytosis in people with T1D. Total lymphocytes, including $CD3^+$ T cells are mobilised in both T1D and control participants. This agrees with previous studies where vigorous exercise induces a significant rise in peripheral blood lymphocytes, followed by lymphopenia, in healthy cohorts [49, 57-59, 82]. Within the T cell compartment, $CD4^+$ T cells mobilised during vigorous exercise in the control group, but not the T1D group. This had a downstream effect on $CD4^+$ T cell subsets in T1D participants because no significant mobilisation of differentiated $CD4^+$ T cell lineage subsets was observed during exercise. In agreement with previous studies, the changes seen during exercise within the $CD4^+$ compartment were minimal compared to changes seen within the $CD8^+$ compartment [51, 52].

Vigorous intensity exercise significantly mobilised $CD8^+$ T cells in both T1D and control participants. $CD8^+$ T cells increased to the same extent after vigorous intensity exercise in both the T1D and control group. As reported in previous studies, $CD8^+$ EMRA were the most sensitive T cell subset to mobilisation, and are shown to mobilise by vigorous exercise in this study herein and others [51-53]. However, the percentage increase of $CD8^+$ EMRA in the T1D group following vigorous intensity exercise was blunted. Further phenotyping of $CD8^+$ EMRA populations revealed that the mobilised subsets mainly comprised of fully differentiated ($CD27^-CD28^-$), recently activated tissue-resident ($CD69^+$) EMRA with migratory capacity ($CD11b^+$). Again, the percentage increase of these $CD8^+$ EMRA subsets following vigorous exercise was blunted in the T1D group. To the best of our knowledge, this is the first time this effect has been described in T1D.

Furthermore, the effects of exercise on T_{SCM} subsets have not been previously examined. This is the first time this has been investigated in T1D and healthy cohorts. Although no significant changes over time for either $CD4^+$ or $CD8^+$ T_{SCM} were found, a trend to increase following vigorous intensity exercise as shown by percentage increase was observed in healthy participants. Furthermore, an impaired response in T1D was noted for $CD4^+$ and $CD8^+$ T_{SCM} subsets, with both subsets decreasing following vigorous intensity exercise.

In this study, we used additional cell surface markers to define the function and fate of $CD4^+$ and $CD8^+$ T cell lineage subsets. Increases in both CD69 and CD11b on $CD4^+$ and $CD8^+$ lymphocytes have been reported following exercise previously, but not on specific T cell subsets as demonstrated in this study herein [58, 83-91]. An increase in $CD4^+$ and $CD8^+$ T cell lineage subsets expressing CD69 is evident in both groups, with a larger percentage increase post-exercise observed in healthy participants. $CD4^+$ and $CD8^+$ T cell lineage subsets expressing CD11b is also noted following exercise in both groups. However, the percentage increase post-exercise in the T1D participants is smaller. $CD95^+$ memory T cells are known to mobilise following vigorous intensity exercise in healthy participants [61, 64]. In our study, $CD95^+$ EM and EMRA T cells from both the $CD4^+$ and $CD8^+$ compartments increased immediately post-exercise, however this increase did not reach statistical significance. Again, it is evident that this response is blunted in T1D as the percentage increase following vigorous intensity exercise is much lower than that of the control group.

There are a number of potential reasons for this blunted response in T1D. Firstly, blood glucose levels are elevated in people with T1D (above 5mM) during and following. High glucose conditions affect the mobilisation of several cell types and therefore may affect lymphocytosis. Elevated plasma glucose in T1D and T2D rodent models have been reported to impair $CD34^+$ HSPC, $CD45^+$, and fibroblast cell migration [92, 93]. Furthermore, fewer lymphocytes were found in the wound site of streptozotocin-induced diabetic mice compared to control, suggesting lower migration of lymphocytes in T1D [94]. Therefore, high glucose levels in T1D may have an effect on T cell migration during exercise.

Secondly, the T cell subsets which exhibit blunted lymphocytosis during vigorous exercise in T1D are those which typically exhibit a high level of beta-adrenergic receptor expression. It is recognised that catecholamine responses can be blunted in T1D. Natural killer (NK) cells and cytotoxic T lymphocytes express much higher levels of beta-adrenergic receptors than other mononuclear cells, causing their dramatic mobilisation during exercise [95-98]. Alterations in these receptors in T1D would alter the stress response to exercise. Reduced beta-adrenergic sensitivity of lymphocytes in T1D has been reported, resulting in a dampened adrenaline response [99-101]. During acute exercise, increased beta-adrenoceptor density and sensitivity of lymphocytes is noted in healthy participants. However, patients with congestive heart failure (CHF) exhibited a blunted increase in beta-adrenoceptor density and no increase in sensitivity [102]. A similar effect may be seen in T1D and may impact exercise-induced lymphocytosis. A limitation to our study is that we did not measure plasma epinephrine, norepinephrine or cortisol which would offer insight into the adrenaline response following acute exercise in people with T1D.

Lastly, the T cell subsets exhibiting blunted lymphocytosis during vigorous exercise share characteristics with those sequestered in the T1D pancreas [6, 7, 103, 104]. These include $CD8^+CD69^+$ T cells [103, 105], $CD8^+CD11b^+$ T cells [6, 7], and highly differentiated memory T cells [2, 4, 8]. The proportion of $CD8^+CD69^+$ T cells was higher in the pancreas than salivary glands taken from NOD mice [103]. $CD8^+CD11b^+$ T cells have also been found within NOD islets [6, 7] and found to be higher in the islets of NOD mice compared to peripheral blood [104]. Furthermore, antigen-experienced islet reactive $CD8^+$ T cells were found sequestered in the pancreas of T1D donors [4]. Islet reactive $CD8^+$ T cells have been shown to exhibit a highly differentiated memory phenotype [2, 8], similar to the $CD8^+$ EMRA T cell phenotype. More recently, islet specific $CD8^+$ T cells were shown to display a T_{SCM} phenotype. In our study, lymphocytosis of highly differentiated $CD8^+$ EMRA that express CD69 and CD11b was blunted immediately following vigorous exercise in T1D. Both $CD4^+$ and $CD8^+$ T_{SCM} also displayed a blunted percentage increase following vigorous exercise compared to the control group. It therefore is possible to postulate that the blunted increase following exercise in these $CD8^+$ T cell subsets is due to their sequestration in the pancreas in T1D.

The results of our study have a number of implications for T1D. Firstly, this study provides imperative evidence of the effects of acute exercise on immunity in people with T1D. Importantly, this provides a platform for future investigations of exercise in T1D, allowing for new avenues to be explored to increase the initial honeymoon phase following diagnosis [106], reduce disease severity, and ultimately for the treatment of T1D. Acute exercise has the potential to regulate immunity in T1D through increased immunosurveillance, deletion of islet reactive T cells and thereby creation of immune space. Lymphocyte trafficking and tissue redistribution is essential for immunosurveillance and regulation [59, 60]. The deletion of exercise sensitive EM and EMRA $CD8^+$ T cells following acute exercise has the potential to regulate immunity through the creation of "immunological space" in people with T1D [61-66]. This immune space following exercise could be taken up by newly generated HSPC and reprogram immune memory [67, 68], thereby reducing aggressive memory T cell phenotypes and ultimately modulating beta cell autoimmunity in T1D.

Furthermore, acute exercise could be used in combination with current trial immunotherapies, and possibly boost the response to immunotherapeutic agents, which to date have been lacking for treatment of T1D. Some immunotherapeutic approaches to modulate beta cell autoimmunity in T1D aim to reduce aggressive memory phenotypes and promote the generation of new naïve cells in T1D. This has been achieved previously with both acute exercise and exercise training in healthy cohorts. Therefore, exercise in T1D may be used as an adjunct for other immunotherapeutic agents. One such immunotherapy, teplizumab, a nonactivating anti-CD3 monoclonal antibody, aims to reduce effector memory subsets, and increase naïve and early memory subsets. This could potentially also be achieved solely by an acute exercise bout or used to boost target T cell populations by administering the treatment directly after an acute exercise bout. Anti-CD3 mAbs that are non-Fc receptor (FcR) binding, like teplizumab, selectively induce apoptosis of antigen-activated T cell phenotypes such as those with memory/pathogenic phenotypes but not naïve T cells [107]. Teplizumab treatment resulted in maintained or improved beta cell function for at least 2 years post treatment in recent-onset T1D patients [108-111]. Evidence for beta cell preservation was also reported in long standing T1D patients up to 1 year post clinical diagnosis [112]. One study identified a group of responders who had higher activated $CD8^+$ terminally differentiated effector and $CD8^+$ EM in T cells at baseline [113]. This is consistent with increased $CD8^+$ terminally differentiated EM T cells following acute exercise. Therefore, frequent bouts of acute exercise could create an environment in which T1D participants respond better to treatment.

The reduced T cell mobilisation seen in T1D may also contribute to the three-fold increased susceptibility to infection associated with this condition [114]. Mobilisation is an important aspect of surveillance for infection and a reduced ability for T cells to mobilise could potentially contribute to a poorer handling of bacterial infections.

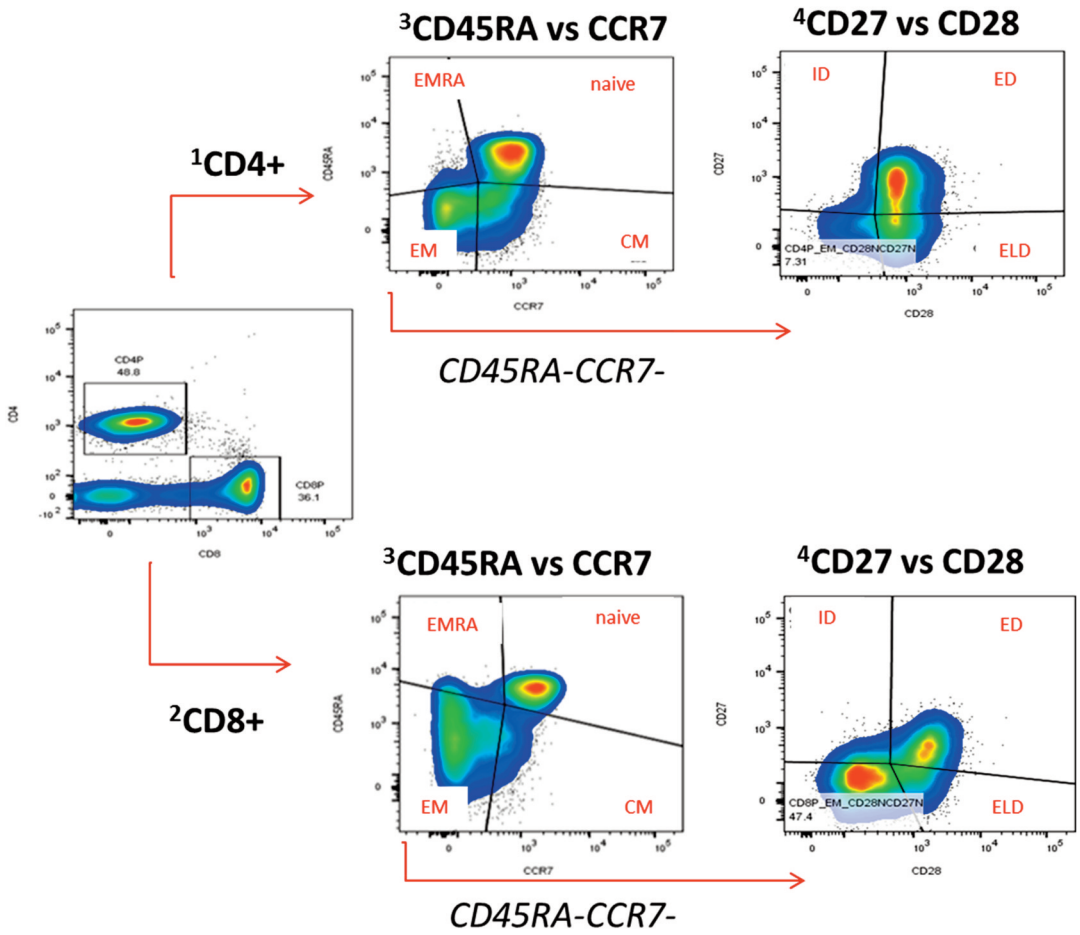
Preliminary data from this study herein also provides a basis to investigate exercise training (as opposed to a single bout of exercise) in T1D. Chronic exercise reduces senescent T cells in the blood [115]. In an ageing study, physically fit age matched controls had a lower proportion of peripheral blood memory T cells ($KLRG1^+CD57^+$, $KLRG1^+CD28^-$) compared to those with a lower VO_2 max [66, 116]. Naïve-memory T cell balance is disrupted in the ageing population and a similar observation has been found in T1D [117]. Therefore, reduced senescent memory T cell populations may also occur from exercise training in T1D.

The mechanisms by which exercise training could preserve beta cells in T1D have yet to be explored. Evidence of immunomodulation by exercise training in T1D is limited. Two studies to date have examined exercise training in T1D mouse models. The first study showed that 6 weeks of exercise training in streptozotocin-induced T1D mice significantly improved insulin content and insulin secretion in islets compared to sedentary mice, suggesting a protective effect against the destruction of the remaining beta cells or the generation of new beta cells [69]. A more recent study has examined the effects of exercise training on immune parameters in T1D [70]. Twenty weeks of training in non-obese diabetic (NOD) mice resulted in reduced immune cell infiltration into the pancreas and subsequently the insulitis index. This is the only exercise study in a model of T1D to demonstrate the modulatory effects of exercise on islet immunity [70].

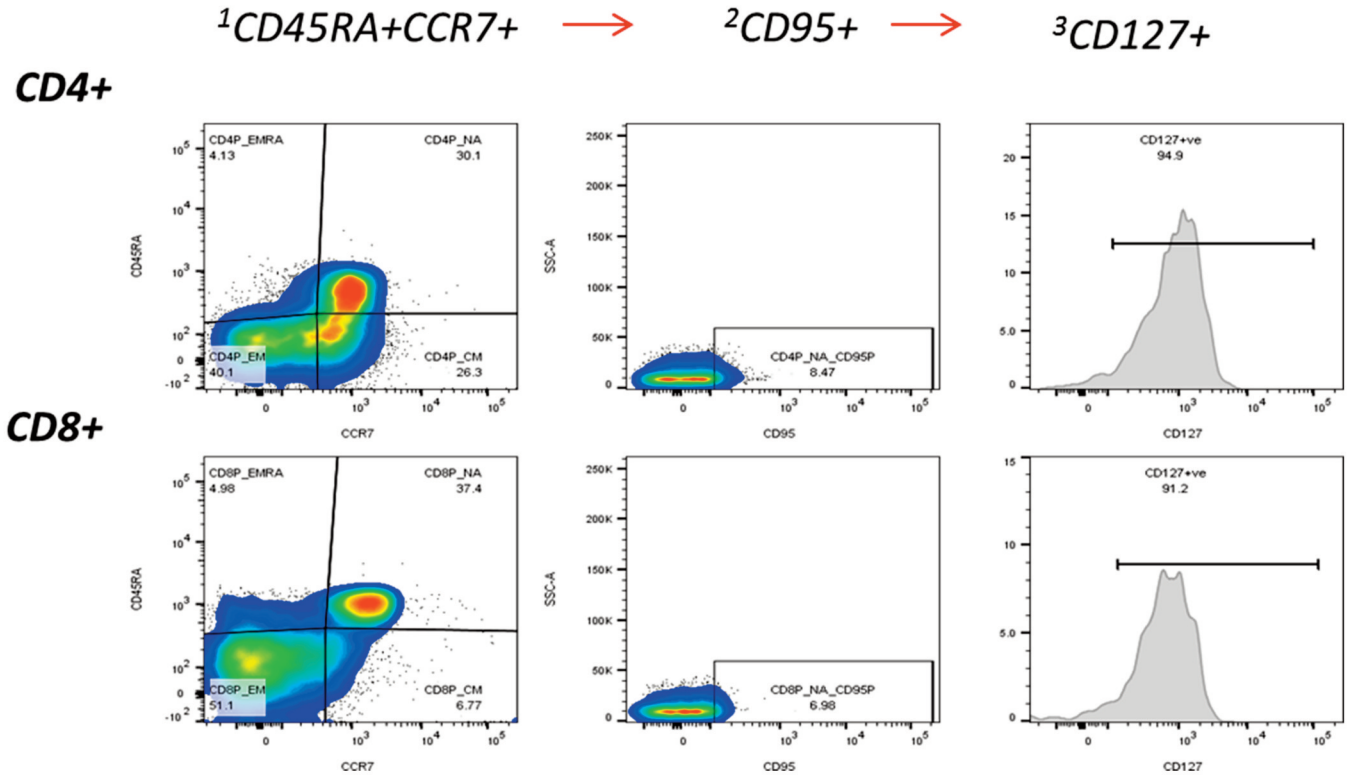
Although not well studied in T1D, exercise training has been demonstrated to modulate immunity in other T cell mediated autoimmune disorders [118]. Mice with EAE, a model for multiple sclerosis (MS), underwent 6 weeks exercise training. T cells taken from lymph nodes had an inhibited immune response to autoantigen whilst sustaining an increased immune response to non-specific stimulus such as concanavalin A [118]. This suggests the generation of new naïve and early memory T cells with exercise training as the recall response to autoantigen is reduced. Another study showed improved immune modulation by exercise in EAE models resulting in delayed onset of disease and increased T cells with a regulatory phenotype [119]. However, other autoimmune disorders such as rheumatoid arthritis (RA) and systemic lupus (SLE) have different responses to exercise, where $CD8^+$ T cells are reduced following exercise and $CD4^+$ T cells are reduced at peak exercise but increased after cessation [120]. It is unclear if exercise training (as opposed to a single bout of exercise) would result in responses to exercise that are comparable to healthy participants.

In conclusion, we show for the first time that acute exercise preferentially mobilises differentiated and antigen experienced $CD8^+$ T cells in T1D, but to a lesser extent than in healthy individuals. A contributing factor to the relatively reduced mobilisation pattern in T1D was attributable to a blunted response among highly differentiated $CD8^+$ T cells, which may indicate sequestering of $CD8^+$ T cells in the pancreas. These findings need to be extended and investigated in an exercise training programme, and the functional implications of this effect on beta cell function explored in a formal clinical trial.

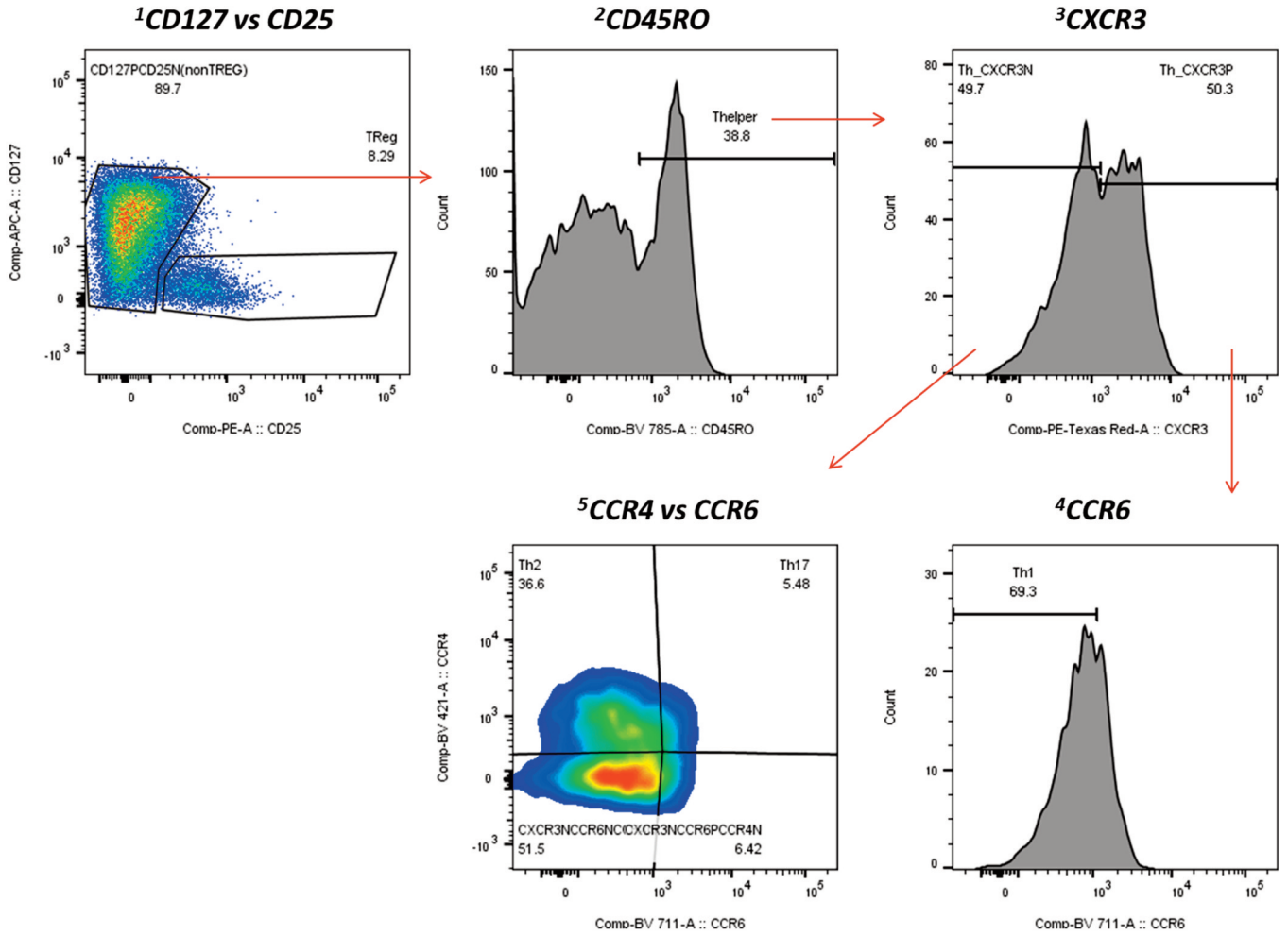
We have previously hypothesised that an exercise training programme has the potential to modulate beta cell loss in people newly diagnosed with T1D [121]. We have tested this hypothesis in a pilot randomised controlled trial [122, 123]. This study showed that beta cell function, when corrected for the changes in insulin sensitivity that accompany physical exercise, appears to be preserved in people with T1D. The results of this most recent work exploring the effect of a single bout of exercise on T cell mobilisation provides mechanistic insight into how exercise may bring about a benefit to beta cell health in people newly diagnosed with T1D. Further evaluation of the immunomodulatory effects of acute exercise on autoreactive memory T cells in T1D is warranted to ascertain impact on disease prognosis. These findings need to be validated in an exercise training study.



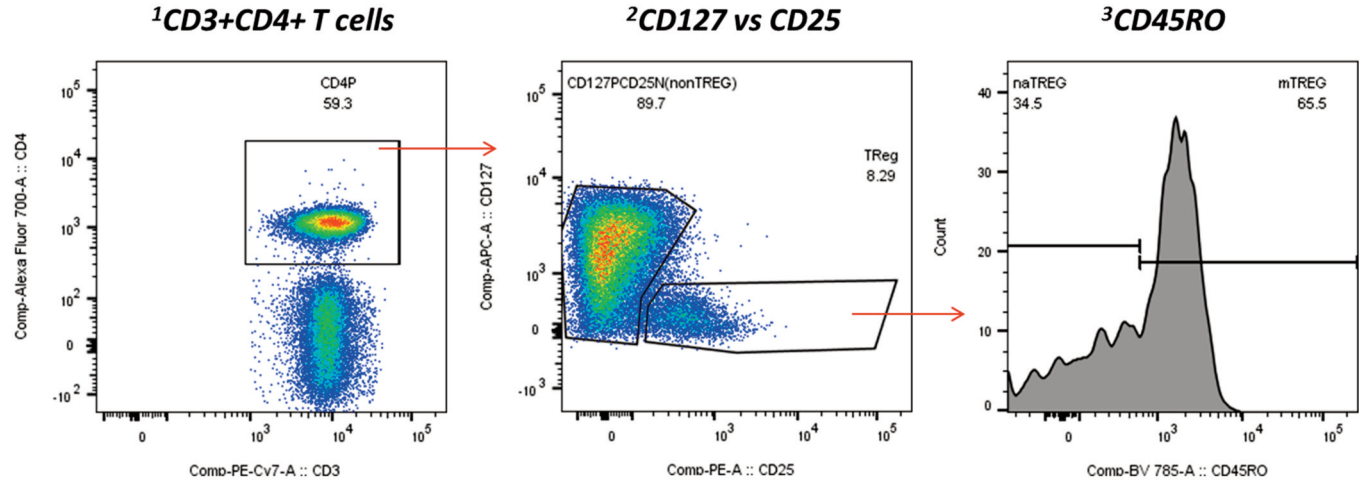
Supplementary Figure 1 CD4 and CD8 T cell sub populations gating strategy shown by representative flow plots.
Representative flow cytometry plots showing following parent gate for CD3+ T cell populations (shown in figure 2) *(1)* CD3+CD4+ T cells gate *(2)* CD3+CD8+ T cells gate *(3)* Quadrant gating for naïve (CD45RA+ CCR7+), central memory (CM: CD45RA- CCR7+), effector memory (EM: CD45RA- CCR7-), and EMRA (CD45RA- CCR7-) *(4)* CD45RA- CCR7- selected to gate on early differentiated (ED; CD45RA- CCR7- CD27+CD28+), early-like differentiated (ELD: CD45RA- CCR7- CD27-CD28+) and intermediately differentiated (ID; CD45RA- CCR7- CD27+CD28-) populations.



Supplementary Figure 2 T_{SCM} gating strategy shown by representative flow plots
Representative flow cytometry plots showing following parent gate for CD3+, CD4+ and CD8+ T cell populations (shown in figure 2 and 3) *(1)* CD45RA+ CCR7+ T cell gate *(2)* CD45RA+ CCR7+CD95+ T cell gate *(3)* T_{SCM} selection (CD45RA+ CCR7+ CD95+ CD127+).



Supplementary Figure 3 CD4 T-helper cell gating strategy shown by representative flow plots.
Representative flow cytometry plots showing following parent gate for CD3+CD4+ T-helper populations (shown in figure 1) *(1)* CD127+CD25- non-TReg gate *(2)* CD45RO+ T-helper cell gate *(3)* CXCR3+ gate for Th1 subsets, CXCR3- gate for Th2/Th17 subsets *(4)* CXCR3+CCR6- Th1 cells *(5)* Quadrant gate used to define CXCR3-CCR4+CCR6-Th2 cells and CXCR3-CCR4+CCR6+ Th17 cells.



Supplementary Figure 4 T-Regulatory cell gating strategy shown by representative flow plots
Representative flow cytometry plots showing following parent gate for CD3+CD4+ T-Regulatory cell populations *(1)* CD3+CD4+ TReg cells gate *(2)* CD127^{low}CD25^{hi} TReg cell gate *(3)* nTReg (CD45RO-) and mTReg (CD45RO+) distinction.

Abbreviations

BMI	Body Mass Index
BP	Blood Pressure
CA^{2+}	Calcium
CCL	Chemokine Ligand
CCR	Chemokine Receptor
CD	Cluster of Differentiation
CMV	Cytomegalovirus
CTL	Cytotoxic T Lymphocyte
CXCR	C-X-C chemokine receptor
ddH2O	Double-distilled water
ED	Early Differentiated
EDTA	Ethylenediaminetetraacetic Acid
ELD	Early-like Differentiated
EM	Effector Memory
EMRA	Effector Memory re-expressing CD45RA
EXTOD	Exercise for Type One Diabetes
FBS	Fetal Bovine Serum
FcR	Fc Receptor
FMO	Fluorescence Minus One
FoxP3	Forkhead winged helix transcription factor
FSC-A	Forward Scatter-Area
FSC-H	Forward Scatter-Height
HbA1c	Haemoglobin A1c
HSPC	Hematopoietic Stem and Progenitor Cells
ID	Intermediately Differentiated
KLRG1	Killer cell Lectin-like Receptor subfamily G member 1
mAb	Monoclonal antibody
mTReg	memory T-Regulatory
naTReg	Naïve T-Regulatory
NK	Natural Killer
O2	Oxygen
PBS	Phosphate Buffer Saline
pTReg	periphery T-Regulatory cell
REC	Research Ethics Committee
S1PR	Sphingosine-1-Phosphate Receptor
sIL2RA	soluble IL2RA
sIL-6R	soluble IL-6R
SSC-A	Side Scatter- Area
SSC-H	Side Scatter- Height
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
Th1	Type 1 helper
Th2	Type 2 helper
Th17	Type 17 helper
TIGIT	T cell immunoreceptor with Ig and ITIM domains
TReg	T-Regulatory
T_{SCM}	Stem cell like memory T cells
UHBFT	University Hospitals Birmingham NHS Foundation Trust
WTCRF	Wellcome Trust Clinical Research Facility
ZnT8	Zinc Transporter

REFERENCES

1. Matteucci, E., et al., Altered Proportions of Naïve, Central Memory and Terminally Differentiated Central Memory Subsets among CD4+ and CD8+ T Cells Expressing CD26 in Patients with Type 1 Diabetes. Journal of Clinical Immunology, 2011. 31(6): p. 977-984.
2. Skowera, A., et al., beta-cell-specific CD8 T cell phenotype in type 1 diabetes reflects chronic autoantigen exposure. Diabetes, 2015. 64(3): p. 916-25.
3. Vignali, D., et al., Detection and Characterization of CD8(+) Autoreactive Memory Stem T Cells in Patients With Type 1 Diabetes. Diabetes, 2018. 67(5): p. 936-945.
4. Culina, S., et al., Islet-reactive CD8(+) T cell frequencies in the pancreas, but not in blood, distinguish type 1 diabetic patients from healthy donors. Sci Immunol, 2018. 3(20).
5. Radenkovic, M., et al., Characterization of resident lymphocytes in human pancreatic islets. Clin Exp Immunol, 2017. 187(3): p. 418-427.
6. Calderon, B., et al., Entry of diabetogenic T cells into islets induces changes that lead to amplification of the cellular response. Proc Natl Acad Sci U S A, 2011. 108(4): p. 1567-72.
7. Goldrath, A.W., et al., Differences in adhesion markers, activation markers, and TcR in islet infiltrating vs. peripheral lymphocytes in the NOD mouse. J Autoimmun, 1995. 8(2): p. 209-20.
8. Monti, P., et al., Evidence for in vivo primed and expanded autoreactive T cells as a specific feature of patients with type 1 diabetes. J Immunol, 2007. 179(9): p. 5785-92.
9. Miller, J.F.A.P., IMMUNOLOGICAL FUNCTION OF THE THYMUS. The Lancet. 278(7205): p. 748-749.
10. Cossarizza, A., et al., Guidelines for the use of flow cytometry and cell sorting in immunological studies. European Journal of Immunology, 2017. 47(10): p. 1584-1797.
11. Maecker, H.T., J.P. McCoy, and R. Nussenblatt, Standardizing immunophenotyping for the Human Immunology Project. Nat Rev Immunol, 2012. 12(3): p. 191-200.
12. Larbi, A. and T. Fulop, From "truly naïve" to "exhausted senescent" T cells: When markers predict functionality. Cytometry Part A, 2014. 85(1): p. 25-35.
13. Sallusto, F., et al., Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature, 1999. 401(6754): p. 708-12.
14. Sanders, M.E., M.W. Makgoba, and S. Shaw, Human naive and memory T cells: reinterpretation of helper-inducer and suppressor-inducer subsets. Immunology Today, 1988. 9(7): p. 195-199.
15. Barclay, A.N., et al., The leukocyte-common antigen (L-CA) family. Adv Exp Med Biol, 1988. 237: p. 3-7.
16. Akbar, A.N., et al., Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells. J Immunol, 1988. 140(7): p. 2171-8.
17. Hamann, D., et al., Evidence that human CD8+CD45RA+CD27- cells are induced by antigen and evolve through extensive rounds of division. Int Immunol, 1999. 11(7): p. 1027-33.
18. Borthwick, N.J., et al., Loss of CD28 expression on CD8(+) T cells is induced by IL-2 receptor gamma chain signalling cytokines and type I IFN, and increases susceptibility to activation-induced apoptosis. Int Immunol, 2000. 12(7): p. 1005-13.

19. Tomiyama, H., T. Matsuda, and M. Takiguchi, Differentiation of Human CD8⁺ T Cells from a Memory to Memory/Effector Phenotype. The Journal of Immunology, 2002. 168(11): p. 5538-5550.
20. Tomiyama, H., T. Matsuda, and M. Takiguchi, Differentiation of human CD8(+) T cells from a memory to memory/effector phenotype. J Immunol, 2002. 168(11): p. 5538-50.
21. Michie, C.A., et al., Lifespan of human lymphocyte subsets defined by CD45 isoforms. Nature, 1992. 360: p. 264.
22. Arlettaz, L., et al., CD45 isoform phenotypes of human T cells: CD4(+)CD45RA(-)RO(+) memory T cells re-acquire CD45RA without losing CD45RO. Eur J Immunol, 1999. 29(12): p. 3987-94.
23. Faint, J.M., et al., Memory T Cells Constitute a Subset of the Human CD8⁺CD45RA⁺ Pool with Distinct Phenotypic and Migratory Characteristics. The Journal of Immunology, 2001. 167(1): p. 212-220.
24. Romero, P., et al., Four functionally distinct populations of human effector-memory CD8+ T lymphocytes. J Immunol, 2007. 178(7): p. 4112-9.
25. Koch, S., et al., Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people. Immunity & Ageing : I & A, 2008. 5: p. 6-6.
26. Gattinoni, L., et al., A human memory T cell subset with stem cell-like properties. Nat Med, 2011. 17(10): p. 1290-7.
27. Ahmed, R., et al., Human Stem Cell-like Memory T Cells Are Maintained in a State of Dynamic Flux. Cell Reports, 2016. 17(11): p. 2811-2818.
28. Gattinoni, L., et al., A human memory T-cell subset with stem cell-like properties. Nature medicine, 2011. 17(10): p. 1290-1297.
29. Lugli, E., et al., Superior T memory stem cell persistence supports long-lived T cell memory. J Clin Invest, 2013. 123(2): p. 594-9.
30. Zhu, J., H. Yamane, and W.E. Paul, Differentiation of effector CD4 T cell populations (*). Annu Rev Immunol, 2010. 28: p. 445-89.
31. Glimcher, L.H. and K.M. Murphy, Lineage commitment in the immune system: the T helper lymphocyte grows up. Genes Dev, 2000. 14(14): p. 1693-711.
32. Mosmann, T.R., et al., Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. The Journal of Immunology, 1986. 136(7): p. 2348-2357.
33. Mosmann, T.R. and R.L. Coffman, TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol, 1989. 7: p. 145-73.
34. Duhen, T., et al., Functionally distinct subsets of human FOXP3+ Treg cells that phenotypically mirror effector Th cells. Blood, 2012. 119(19): p. 4430-40.
35. Qin, S., et al., The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. The Journal of Clinical Investigation, 1998. 101(4): p. 746-754.
36. Hirahara, K., et al., Mechanisms Underlying Helper T cell Plasticity: Implications for Immune-mediated Disease. The Journal of allergy and clinical immunology, 2013. 131(5): p. 1276-1287.
37. Acosta-Rodriguez, E.V., et al., Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. Nat Immunol, 2007. 8(6): p. 639-46.
38. Harrington, L.E., et al., Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol, 2005. 6(11): p. 1123-32.
39. Park, H., et al., A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol, 2005. 6(11): p. 1133-41.
40. Booth, N.J., et al., Different proliferative potential and migratory characteristics of human CD4+ regulatory T cells that express either CD45RA or CD45RO. J Immunol, 2010. 184(8): p. 4317-26.
41. Rosenblum, M.D., et al., Response to self antigen imprints regulatory memory in tissues. Nature, 2011. 480(7378): p. 538-42.
42. Rosenblum, M.D., S.S. Way, and A.K. Abbas, Regulatory T cell memory. Nature reviews. Immunology, 2016. 16(2): p. 90-101.
43. Joller, N., et al., Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses. Immunity, 2014. 40(4): p. 569-81.
44. Arif, S., et al., Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. J Clin Invest, 2004. 113(3): p. 451-63.
45. Reinert-Hartwall, L., et al., Th1/Th17 Plasticity Is a Marker of Advanced β Cell Autoimmunity and Impaired Glucose Tolerance in Humans. The Journal of Immunology Author Choice, 2015. 194(1): p. 68-75.
46. Garg, G., et al., Type 1 Diabetes-Associated IL2RA Variation Lowers IL-2 Signaling and Contributes to Diminished CD4⁺CD25⁺ Regulatory T Cell Function. The Journal of Immunology, 2012. 188(9): p. 4644-4653.
47. Campbell, J.P. and J.E. Turner, Debunking the Myth of Exercise-Induced Immune Suppression: Redefining the Impact of Exercise on Immunological Health Across the Lifespan. 2018. 9(648).
48. Gannon, G.A., et al., Differential cell adhesion molecule expression and lymphocyte mobilisation during prolonged aerobic exercise. Eur J Appl Physiol, 2001. 84(4): p. 272-82.
49. Shek, P.N., et al., Strenuous exercise and immunological changes: a multiple-time-point analysis of leukocyte subsets, CD4/CD8 ratio, immunoglobulin production and NK cell response. Int J Sports Med, 1995. 16(7): p. 466-74.
50. Pizza, F.X., et al., Exercise-induced muscle damage: effect on circulating leukocyte and lymphocyte subsets. Med Sci Sports Exerc, 1995. 27(3): p. 363-70.
51. Spielmann, G., et al., A single exercise bout enhances the manufacture of viral-specific T-cells from healthy donors: implications for allogeneic adoptive transfer immunotherapy. Scientific Reports, 2016. 6: p. 25852.
52. Campbell, J.P., et al., Acute exercise mobilises CD8+ T lymphocytes exhibiting an effector-memory phenotype. Brain Behav Immun, 2009. 23(6): p. 767-75.
53. Turner, J.E., et al., Latent cytomegalovirus infection amplifies CD8 T-lymphocyte mobilisation and egress in response to exercise. Brain Behav Immun, 2010. 24(8): p. 1362-70.
54. Simpson, R.J., et al., Senescent phenotypes and telomere lengths of peripheral blood T-cells mobilized by acute exercise in humans. Exerc Immunol Rev, 2010. 16: p. 40-55.
55. Simpson, R.J., et al., Senescent T-lymphocytes are mobilised into the peripheral blood compartment in young and older humans after exhaustive exercise. Brain Behav Immun, 2008. 22(4): p. 544-51.

56. Bruunsgaard, H., et al., Exercise induces recruitment of lymphocytes with an activated phenotype and short telomeres in young and elderly humans. Life Sci, 1999. 65(24): p. 2623-33.
57. Gabriel, H., A. Urhausen, and W. Kindermann, Mobilization of circulating leucocyte and lymphocyte subpopulations during and after short, anaerobic exercise. European Journal of Applied Physiology and Occupational Physiology, 1992. 65(2): p. 164-170.
58. Simpson, R.J., et al., The effects of intensive, moderate and downhill treadmill running on human blood lymphocytes expressing the adhesion/activation molecules CD54 (ICAM-1), CD18 (beta2 integrin) and CD53. Eur J Appl Physiol, 2006. 97(1): p. 109-21.
59. Kruger, K. and F.C. Mooren, T cell homing and exercise. Exerc Immunol Rev, 2007. 13: p. 37-54.
60. Kruger, K., et al., Exercise-induced redistribution of T lymphocytes is regulated by adrenergic mechanisms. Brain Behav Immun, 2008. 22(3): p. 324-38.
61. Mooren, F.C., et al., Lymphocyte apoptosis after exhaustive and moderate exercise. J Appl Physiol (1985), 2002. 93(1): p. 147-53.
62. Hoffman-Goetz, L. and J. Quadrilatero, Treadmill exercise in mice increases intestinal lymphocyte loss via apoptosis. Acta Physiol Scand, 2003. 179(3): p. 289-97.
63. Mars, M., et al., High Intensity Exercise: A Cause of Lymphocyte Apoptosis? Biochemical and Biophysical Research Communications, 1998. 249(2): p. 366-370.
64. Timmons, B.W. and O. Bar-Or, Lymphocyte expression of CD95 at rest and in response to acute exercise in healthy children and adolescents. Brain Behav Immun, 2007. 21(4): p. 442-9.
65. Kruger, K. and F.C. Mooren, Exercise-induced leukocyte apoptosis. Exerc Immunol Rev, 2014. 20: p. 117-34.
66. Simpson, R.J., Aging, persistent viral infections, and immunosenescence: can exercise "make space"? Exerc Sport Sci Rev, 2011. 39(1): p. 23-33.
67. Baker, J.M., J.P. Nederveen, and G. Parise, Aerobic exercise in humans mobilizes HSCs in an intensity-dependent manner. J Appl Physiol (1985), 2017. 122(1): p. 182-190.
68. Emmons, R., et al., Acute exercise mobilizes hematopoietic stem and progenitor cells and alters the mesenchymal stromal cell secretome. Journal of Applied Physiology, 2016. 120(6): p. 624-632.
69. Huang, H.H., et al., Exercise increases insulin content and basal secretion in pancreatic islets in type 1 diabetic mice. Exp Diabetes Res, 2011. 2011: p. 481427.
70. Oharomari, L.K., C. de Moraes, and A.M. Navarro, Exercise Training but not Curcumin Supplementation Decreases Immune Cell Infiltration in the Pancreatic Islets of a Genetically Susceptible Model of Type 1 Diabetes. Sports Medicine - Open, 2017. 3: p. 15.
71. Cohen, J., et al., Sex steroid receptors in peripheral T cells: absence of androgen receptors and restriction of estrogen receptors to OKT8-positive cells. The Journal of Immunology, 1983. 131(6): p. 2767-2771.
72. Liu, H.Y., et al., Estrogen inhibition of EAE involves effects on dendritic cell function. Journal of neuroscience research, 2002. 70(2): p. 238-248.
73. González, D.A., et al., Sex hormones and autoimmunity. Immunology letters, 2010. 133(1): p. 6-13.
74. Cink, R.E. and T.R. Thomas, Validity of the Astrand-Ryhming nomogram for predicting maximal oxygen intake. British Journal of Sports Medicine, 1981. 15(3): p. 182-185.
75. Rooney, B.V., et al., Lymphocytes and monocytes egress peripheral blood within minutes after cessation of steady state exercise: A detailed temporal analysis of leukocyte extravasation. Physiol Behav, 2018. 194: p. 260-267.
76. Hagstromer, M., P. Oja, and M. Sjostrom, The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. Public Health Nutr, 2006. 9(6): p. 755-62.
77. Clements, K. and G. Turpin, The life events scale for students: Validation for use with British samples. Personality and Individual Differences, 1996. 20(6): p. 747-751.
78. Cohen, S., T. Kamarck, and R. Mermelstein, A global measure of perceived stress. J Health Soc Behav, 1983. 24(4): p. 385-96.
79. Crandall, C.S., J.J. Preisler, and J. Aussprung, Measuring life event stress in the lives of college students: the Undergraduate Stress Questionnaire (USQ). J Behav Med, 1992. 15(6): p. 627-62.
80. Marmot, M.G., et al., Health inequalities among British civil servants: the Whitehall II study. Lancet, 1991. 337(8754): p. 1387-93.
81. Buysse, D.J., et al., The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res, 1989. 28(2): p. 193-213.
82. Walsh, N.P., et al., Position statement. Part one: Immune function and exercise. Exerc Immunol Rev, 2011. 17: p. 6-63.
83. Gabriel, H., et al., Increased CD45RA+CD45RO+ cells indicate activated T cells after endurance exercise. Med Sci Sports Exerc, 1993. 25(12): p. 1352-7.
84. Morabito, C., et al., Responses of peripheral blood mononuclear cells to moderate exercise and hypoxia. Scand J Med Sci Sports, 2016. 26(10): p. 1188-99.
85. Christensen, J.E., et al., CD11b expression as a marker to distinguish between recently activated effector CD8(+) T cells and memory cells. Int Immunol, 2001. 13(4): p. 593-600.
86. Muto, S., V. Vetvicka, and G.D. Ross, CR3 (CD11b/CD18) expressed by cytotoxic T cells and natural killer cells is upregulated in a manner similar to neutrophil CR3 following stimulation with various activating agents. J Clin Immunol, 1993. 13(3): p. 175-84.
87. Bevilacqua, M.P., Endothelial-Leukocyte Adhesion Molecules. Annual Review of Immunology, 1993. 11(1): p. 767-804.
88. Hynes, R.O., Integrins: versatility, modulation, and signaling in cell adhesion. Cell, 1992. 69(1): p. 11-25.
89. van Eeden, S.F., et al., Expression of the cell adhesion molecules on leukocytes that demarginate during acute maximal exercise. J Appl Physiol (1985), 1999. 86(3): p. 970-6.
90. Kurokawa, Y., et al., Exercise-induced changes in the expression of surface adhesion molecules on circulating granulocytes and lymphocytes subpopulations. European Journal of Applied Physiology and Occupational Physiology, 1995. 71(2): p. 245-252.
91. Nielsen, H.G. and T. Lyberg, Long-distance running modulates the expression of leucocyte and endothelial adhesion molecules. Scand J Immunol, 2004. 60(4): p. 356-62.

92. Ferraro, F., et al., DIABETES IMPAIRS HEMATOPOIETIC STEM CELL MOBILIZATION THROUGH ALTERATION OF NICHE FUNCTION. Science translational medicine, 2011. 3(104): p. 104ra101-104ra101.
93. Xuan, Y.H., et al., High-Glucose Inhibits Human Fibroblast Cell Migration in Wound Healing via Repression of bFGF-Regulating JNK Phosphorylation. PLoS ONE, 2014. 9(9): p. e108182.
94. Fahey, T.J., III, et al., Diabetes impairs the late inflammatory response to wound healing. Journal of Surgical Research. 50(4): p. 308-313.
95. Maisel, A.S., et al., Beta-adrenergic receptors in lymphocyte subsets after exercise. Alterations in normal individuals and patients with congestive heart failure. Circulation, 1990. 82(6): p. 2003-10.
96. Yu, X.Y., et al., Evidence for coexistence of three beta-adrenoceptor subtypes in human peripheral lymphocytes. Clin Pharmacol Ther, 2007. 81(5): p. 654-8.
97. Dimitrov, S., T. Lange, and J. Born, Selective Mobilization of Cytotoxic Leukocytes by Epinephrine. The Journal of Immunology, 2010. 184(1): p. 503-511.
98. Benschop, R.J., M. Rodriguez-Feuerhahn, and M. Schedlowski, Catecholamine-induced leukocytosis: early observations, current research, and future directions. Brain Behav Immun, 1996. 10(2): p. 77-91.
99. Fritsche, A., et al., Effect of hypoglycemia on beta-adrenergic sensitivity in normal and type 1 diabetic subjects. Diabetes Care, 1998. 21(9): p. 1505-10.
100. Korytkowski, M.T., et al., Reduced beta-adrenergic sensitivity in patients with type 1 diabetes and hypoglycemia unawareness. Diabetes Care, 1998. 21(11): p. 1939-43.
101. Trovik, T.S., et al., Dysfunction in the beta 2-adrenergic signal pathway in patients with insulin dependent diabetes mellitus (IDDM) and unawareness of hypoglycaemia. Eur J Clin Pharmacol, 1995. 48(5): p. 327-32.
102. Mancini, D.M., et al., Characterization of lymphocyte beta-adrenergic receptors at rest and during exercise in ambulatory patients with chronic congestive heart failure. American Journal of Cardiology. 63(5): p. 307-312.
103. Faveeuw, C., M.C. Gagnerault, and F. Lepault, Expression of homing and adhesion molecules in infiltrated islets of Langerhans and salivary glands of nonobese diabetic mice. J Immunol, 1994. 152(12): p. 5969-78.
104. Goldrath, A.W., et al., Differences in Adhesion Markers, Activation Markers, and TcR in Islet Infiltratingvs.Peripheral Lymphocytes in the NOD Mouse. Journal of Autoimmunity, 1995. 8(2): p. 209-220.
105. Magnuson, A.M., et al., Population dynamics of islet-infiltrating cells in autoimmune diabetes. Proc Natl Acad Sci U S A, 2015. 112(5): p. 1511-6.
106. Chetan, M., et al., The Type 1 diabetes 'honeymoon'period is five times longer in men who exercise: a case–control study. Diabetic Medicine, 2018.
107. Carpenter, P.A., et al., Non-Fc receptor-binding humanized anti-CD3 antibodies induce apoptosis of activated human T cells. J Immunol, 2000. 165(11): p. 6205-13.
108. Herold, K.C., et al., Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. N Engl J Med, 2002. 346(22): p. 1692-8.
109. Keymeulen, B., et al., Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. N Engl J Med, 2005. 352(25): p. 2598-608.
110. Sherry, N., et al., Teplizumab for treatment of type 1 diabetes (Protege study): 1-year results from a randomised, placebo-controlled trial. Lancet, 2011. 378(9790): p. 487-97.
111. Hagopian, W., et al., Teplizumab preserves C-peptide in recent-onset type 1 diabetes: two-year results from the randomized, placebo-controlled Protege trial. Diabetes, 2013. 62(11): p. 3901-8.
112. Herold, K.C., et al., Teplizumab treatment may improve C-peptide responses in participants with type 1 diabetes after the new-onset period: a randomised controlled trial. Diabetologia, 2013. 56(2): p. 391-400.
113. Herold, K.C., et al., Teplizumab (anti-CD3 mAb) treatment preserves C-peptide responses in patients with new-onset type 1 diabetes in a randomized controlled trial: metabolic and immunologic features at baseline identify a subgroup of responders. Diabetes, 2013. 62(11): p. 3766-74.
114. Carey, I.M., et al., Risk of Infection in Type 1 and Type 2 Diabetes Compared With the General Population: A Matched Cohort Study. Diabetes Care, 2018. 41(3): p. 513-521.
115. Wang, J.S., W.L. Chen, and T.P. Weng, Hypoxic exercise training reduces senescent T-lymphocyte subsets in blood. Brain Behav Immun, 2011. 25(2): p. 270-8.
116. Spielmann, G., et al., Aerobic fitness is associated with lower proportions of senescent blood T-cells in man. Brain Behav Immun, 2011. 25(8): p. 1521-9.
117. Ilonen, J., H.M. Surcel, and M.L. Kaar, Abnormalities within CD4 and CD8 T lymphocytes subsets in type 1 (insulin-dependent) diabetes. Clin Exp Immunol, 1991. 85(2): p. 278-81.
118. Einstein, O., et al., Exercise training attenuates experimental autoimmune encephalomyelitis by peripheral immunomodulation rather than direct neuroprotection. Exp Neurol, 2018. 299(Pt A): p. 56-64.
119. Souza, P.S., et al., Physical Exercise Attenuates Experimental Autoimmune Encephalomyelitis by Inhibiting Peripheral Immune Response and Blood-Brain Barrier Disruption. Mol Neurobiol, 2017. 54(6): p. 4723-4737.
120. Pool, A.J., et al., Serum cortisol reduction and abnormal prolactin and CD4+/CD8+ T-cell response as a result of controlled exercise in patients with rheumatoid arthritis and systemic lupus erythematosus despite unaltered muscle energetics. Rheumatology (Oxford), 2004. 43(1): p. 43-8.
121. Narendran, P., et al., The time has come to test the beta cell preserving effects of exercise in patients with new onset type 1 diabetes. Diabetologia, 2015. 58(1): p. 10-8.
122. Narendran, P., et al., Exercise to preserve beta-cell function in recent-onset Type 1 diabetes mellitus (EXTOD) - a randomized controlled pilot trial. Diabet Med, 2017. 34(11): p. 1521-1531.
123. Lascar, N., et al., Exercise to preserve beta cell function in recent-onset type 1 diabetes mellitus (EXTOD)--a study protocol for a pilot randomized controlled trial. Trials, 2013. 14: p. 180.

Effects of Aerobic Exercise on Molecular Aspects of Asthma: Involvement of SOCS-JAK-STAT

Almeida-Oliveira AR[1,2*], Aquino-Junior JCJ[1,2*], Abbasi A[3*], Santos-Dias A[2], Oliveira-Junior MC[2], Alberca-Custodio RW[2], Rigonato-Oliveira NC[1], Salles-Dias LP[4], Damaceno-Rodrigues NR[5], Caldini EG[5], Arantes-Costa FM[6], Ligeiro-Oliveira AP[1], Belvisi MG[7], Vieira RP[4,2,8,9]

[1] Nove de Julho University, Rua Vergueiro 235/249, Liberdade, São Paulo – SP, Brazil, 01504-001.
[2] Brazilian Institute of Teaching and Research in Pulmonary and Exercise Immunology (IBEPIPE). Rua Pedro Ernesto 240, São José dos Campos – SP, Brazil, 12245-520.
[3] Division of Respiratory & Critical Care Physiology & Medicine, Department of Medicine, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA 90502, USA.
[4] Universidade Brasil, Post-graduation Program in Bioengineering and in Biomedical Engineering, Campus Itaquera, Rua Carolina Fonseca 235, São Paulo – SP, Brazil, 08230-030.
[5] University of Sao Paulo, School of Medicine, Department of Pathology, Laboratory of Cell Biology (LIM 59), Avenida Doutor Arnaldo 455, Cerqueira Cesar, São Paulo – SP, Brazil, 01246-903.
[6] University of Sao Paulo, School of Medicine, Department of Clinical Medicine, Laboratory of Experimental Therapeutics (LIM 20), Avenida Doutor Arnaldo 455, Cerqueira Cesar, São Paulo – SP, Brazil, 01246-903.
[7] Respiratory Pharmacology Group, Airway Disease, National Heart and Lung Institute, Imperial College London, London, UK.
[8] Post-Graduation Program in Sciences of Human Movement and Rehabilitation, Federal University of Sao Paulo (UNIFESP), Campus Baixada Santista, Av. Ana Costa, 95 - Vila Mathias - Santos/SP - CEP: 11060-001.
[9] Anhembi Morumbi University, School of Medicine, Avenida Deputado Benedito Matarazzo, 4050 - Jardim. Aquários, São José dos Campos - SP, Brazil, 12230-002.
* These authors have equally contributed to this study.

ABSTRACT

Background: *Aerobic training (AT) decreases airway inflammation in asthma, but the underlying cellular and molecular mechanisms are not completely understood. Thus, this study evaluated the participation of SOCS-JAK-STAT signaling in the effects of AT on airway inflammation, remodeling and hyperresponsiveness in a model of allergic airway inflammation.*
Methods: *C57Bl/6 mice were divided into Control (Co), Exercise (Ex), HDM (HDM), and HDM+Exercise (HDM+ Ex). Dermatophagoides pteronyssinus (100ug/mouse) were administered oro-tracheally on days 0, 7, 14, 21, 28, 35, 42 and 49. AT was performed in a treadmill during 4 weeks in moderate intensity, from day 24 until day 52.*
Results: *AT inhibited HDM-induced total cells ($p<0.001$), eosinophils ($p<0.01$), neutrophils ($p<0.01$) and lymphocytes ($p<0.01$) in BAL, and eosinophils ($p<0.01$), neutrophils ($p<0.01$) and lymphocytes ($p<0.01$) in peribronchial space. AT also reduced BAL levels of IL-4 ($p<0.001$), IL-5 ($p<0.001$), IL-13 ($p<0.001$), CXCL1 ($p<0.01$), IL-17 ($p<0.01$), IL-23 ($p<0.05$), IL-33 ($p<0.05$), while increased IL-10 ($p<0.05$). Airway collagen fibers ($p<0.01$), elastic fibers $p<0.01$) and mucin ($p<0.01$) were also reduced by AT. AT also inhibited HDM-induced airway hyperresponsiveness (AHR) to methacholine 6,25mg/ml ($p<0.01$), 12,5mg/mL ($p<0.01$), 25mg/mL ($p<0.01$) and 50mg/mL ($p<0.01$). Mechanistically, AT reduced the expression of STAT6 ($p<0.05$), STAT3 ($p<0.001$), STAT5 ($p<0.01$) and JAK2 ($p<0.001$), similarly by peribronchial leukocytes and by airway epithelial cells. SOCS1 expression ($p<0.001$) was upregulated in leukocytes and in epithelial cells, SOCS2 ($p<0.01$) was upregulated in leukocytes and SOCS3 down-regulated in leukocytes ($p<0.05$) and in epithelial cells ($p<0.001$).*
Conclusions: *AT reduces asthma phenotype involving SOCS-JAK-STAT signaling.*

Key words: asthma, exercise immunology, SOCS, JAK, STAT.

INTRODUCTION

Asthma has now become the most prevalent chronic disease in developed countries and affects over 10% of adults [1,2]. Asthma is a chronic inflammatory airway disease, which involves interactions of genetic and environmental factors, whose consequences leads to cough, wheezing, airway hyperresponsiveness and obstruction, caused by inflammation, mucus overproduction, angiogenesis and airway remodeling [1,2]. The chronic inflammation of respiratory tract in asthma is mediated by the increased expression of multiple inflammatory proteins, including cytokines, chemokines, adhesion molecules, products derived of arachidonic acid and receptors. Several signaling pathways have been proposed to be involved in the pathogenesis of asthma. Among those, SOCS (Suppressor of cytokine signaling protein family), JAK (Janus kinase) and STAT (Signal transducer and activator of tran-

Corresponding author
Prof. Dr. Rodolfo Paula Vieira
Universidade Brasil, Post-graduation Program in Bioengineering, Rua Pedro Ernesto 240, São José dos Campos – SP, Brazil, 12245-520. Phone/FAX: +55 12 3303-8390.
E-mail: rodrelena@yahoo.com.br

scription) signaling pathways seems to have a key role [8-12]. However, the results are still controversial, since that in the asthmatic context; some studies show SOCS proteins inhibiting asthmatic phenotype, while others show SOCS increasing asthmatic phenotype, through JAK/STAT modulation [8-12].
Aerobic exercise, which is the main component of pulmonary rehabilitation programs [13] and improves physical fitness, is a strong and effective therapy for pulmonary diseases including asthma [37]. Beyond that, the participation in a pulmonary rehabilitation program is associated with reduced exacerbation [47]. Aerobic exercise, indeed, at low and moderate intensity, can ameliorate lung inflammatory response in the context of different lung diseases and insults, such as emphysema and chronic obstructive pulmonary disease (COPD) [14, 20-22], pulmonary fibrosis [23,24], acute lung injury/acute respiratory distress syndrome [25-27], air pollution [28,29] and asthma [30-35]. However, specifically for asthma, the possible involvement of SOCS, JAK and STAT signaling in the beneficial effects of aerobic exercise is unknown. Aerobic exercise is capable to modulate SOCS, JAK and STAT signaling in different organs and cells, such as blood, testis, kidney, skeletal muscle, endothelial cells, heart and lungs [14-19, 48]. However, until this moment, a single study has investigated the effects of aerobic exercise on STAT3 expression in the lungs, which was done in the context of an experimental model of COPD [14].

Cellular and molecular mechanisms of asthma

Asthma is characterized by chronic inflammation of the respiratory tract. The disease is associated with acute episodes or simply, exacerbations, when the intensity of this inflammation increases [49]. The majority of asthma patients are atopic and have an allergic pattern of inflammation in their airways, which extends from the trachea down to peripheral airways [50]. Allergic inflammation is driven by CD4+ T-helper 2 (Th2) lymphocytes, which secrete interleukin(IL)-4, IL-5 and IL-13 and is referred as Type 2 (T2) asthma, whereas some asthmatic patients have different pattern of inflammation which is known as non-T2 asthma and is associated with more severe disease [51]. In T2 asthma, which represent the most prevalent form of the diseases is characterized by accumulation of eosinophils, mastocytes, CD4+ T helper cells, while non-T2 asthma, beyond these classical cells involved in the allergic process, also present high accumulation of neutrophils. Asthmatic inflammation results in airway narrowing and airway hyperressponsiveness (AHR) which represent the mains physiological abnormality of asthma [50]. The mechanisms of AHR are still unclear but are probably associated with increased release of pro-inflammatory mediators by inflammatory cells (particularly mast cells), increasing the contractility of airway smooth muscle, increasing the sensitivity of airway sensory nerves resulting in airway narrowing for geometric reasons [50].

The molecular mechanisms involved in the pathogenesis of asthma is still not fully understood, however it seems likely that the inhaled allergens activate mast cells, epithelial cells and dendritic cells to locally release several pro-inflammatory mediators, such as chemokines. Released chemokines (CCL17 and CCL22 from dendritic cells and CCL11 from epithelial cells), recruit inflammatory cells especially TH2 cells from blood to the lungs. TH2 cells have a central role in orchestrating the inflammatory response in allergy through the release of interleukin-4 (IL-4) and IL-13 (which stimulate B cells to synthesize IgE), IL-5 (which is necessary for eosinophilic inflammation) and IL-9 (which stimulates mast-cell proliferation). Mast cells release several bronchoconstrictor mediators, including mast cell tryptase, cysteinyl leukotrienes and prostaglandins. In addition, Th17 cells are also increased in asthmatic patients, preferentially in those with severe asthma. These cells may orchestrate neutrophilic inflammation by inducing the release of CXCL8 from airway epithelial cells [52-53]. Furthermore, asthmatic patients may have reduced number of regulatory T (TReg) cells, which suppress TH2 cells, suggesting further TH2-cell proliferation in asthmatic condition [50].

Exercise-induced asthma

Exercise is one of the most common triggers of bronchospasm in persons with and without asthma. Exercise-induced bronchoconstriction (EIB) is defined as transient, reversible bronchoconstriction that develops after strenuous exercise [54]. It is a heterogeneous syndrome occurring in a variety of settings, ranging from the asymptomatic military recruit (whose condition is detected by diagnostic exercise challenge) to the leisure-time athlete with known asthma to the elite athlete for whom EIB may represent an overuse or injury syndrome [55]. Airway obstruction following exercise was first observed among individuals with underlying asthma from which the term exercise-induced asthma (EIA) was derived. Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, and it is associated with bronchial (or airway) hyperresponsiveness. Similar post-exercise asthma-like symptoms have been observed in persons without the presence of co-existing asthma, particularly in athletes. In this population the phenomenon has been referred to as exercise-induced bronchoconstriction (EIB) [56]. According to work group report of American Academy of Allergy, Asthma and Immunology, EIA represents a distinct clinical category of asthma [56]. In fact, most if not all patients with asthma develop symptoms of asthma after a suitable exercise challenge [56]. Moreover, even cases of asthma in which exercise appears to be the only trigger of bronchial obstruction (pure EIA) may be manifestations of chronic inflammation of the airways [56].

Exercise as an anti-inflammatory therapy for asthma

Recent studies strongly support the notion that exercise intervention improve asthma control in adults [57, 58]. These includes improvements in measures such as lung function and quality of life [59], breathlessness [60], and controller therapy [61] while animal models have shown improvements in airway inflammation [62, 63, 64].

Yet asthma is a chronic inflammatory disease, it is highly suggested that effective anti-inflammatory treatments for asthma should reduce diseases progression and the risk of exacerbations [65]. Although there are now effective medications for controlling asthma, it remains poorly controlled in the community with frequent symptoms and exacerbations [66]. It is clear, therefore, that the development of alternative and effective anti-inflammatory therapies for the treatment of asthma is probably the greatest unmet therapeutic need at present. In health, a large body of evidence demonstrates that

lack of physical activity or fitness is associated with an increased risk of cardiovascular disease, stroke, cancer, diabetes and many other chronic diseases [67, 68]. The studies by our group and the others demonstrate that exercise, specifically, chronic aerobic exercise, at low and moderate intensity, can ameliorate lung inflammatory response in asthma condition [30-35, 69]. In a very recent study, our team demonstrated that aerobic exercise attenuated asthma phenotype through modulation of inflammatory cytokines (e.g. IL-5 and IL-13) as well as Leukotriene pathway (LTA4H, CysLT1 receptor, CysLT2 receptor, LTC4 synthase, and BLT2) in an ovalbumin (OVA) model of asthma (30). LTs are potent pro-inflammatory mediators, involved in several aspects of asthma pathophysiology, including bronchoconstriction, edema formation, mucus hypersecretion, as well as inflammatory cell proliferation, activation, and survival [70-72]. In addition, aerobic exercise attenuates dendritic cell and lymphocyte activation in Ovalbumin (OVA) model of allergic airway inflammation [31]. Dendritic cells are the major antigen-presenting cells in the airways and play critical role in initiation and progression of asthma. These cells release several chemokines (e.g,. CCL17, CCL22) to attract Th2 cells into the airways, serving as major regulators of Th2 immune response in asthma [73]. As mentioned previously, Th2 cells have a central role in orchestrating the inflammatory response in asthma [49]. Aerobic exercise also attenuates lung inflammation through attenuation of OVA-specific IgE and IgG1 titers [32]. Furthermore, exercise increases the concentration and expression of several anti-inflammatory mediators such as IL-10 and IL-1ra in the lungs to attenuate lung inflammation [30-35]. Based on these evidences it can be concluded that aerobic exercise decreases airway inflammation and remodeling in, at least, a murine model of asthma. In addition, the anti-inflammatory effects of aerobic exercise on asthmatic airway inflammation has been demonstrated not only in murine models of asthma, but also in asthmatic individuals [37].

SOCS-JAK-STAT pathway in asthma and the role of exercise

Inflammation is etiologically linked to the pathogenesis of all of most of the chronic diseases, and chronic low-grade systemic inflammation is correlated with disease severity in many [70-73]. Cytokines play a pivotal role in the initiation and development of asthma by regulating the expansion of Th2 cells and by mediating many of the Th2 effector functions that underlie the pathogenic events of an asthmatic response [70-73]. Several studies have recently been performed to elucidate the signaling pathways used by cytokines to mediate their actions. These studies have revealed that cytokine-mediated signals are primarily transduced by the JAK-STAT signaling cascade [8-12, 74-77]. The Janus kinase (JAK)–signal transducer of activators of transcription (STAT) pathway, is now recognized as an evolutionarily conserved signaling pathway employed by diverse cytokines, interferons, growth factors, and related molecules and therefore, involved in inflammatory processes. Jaks (tyrosine kinases) engage with cytokine receptors and mediate tyrosine phosphorylation of their associated receptors and recruited proteins, including STATs [78]. Tyrosine phosphorylated STATs are released from the receptors and form homodimers, which translocate to the nucleus where they bind canonical sequences and modulate transcription [79]. In addition to tyrosine phosphorylation, STATs are serine phosphorylated within their transcriptional activation domain, influencing their transcriptional activation function, stability, and non-canonical functions [78]. STAT proteins are critical mediators of immunity to pathogens. Indeed, inflammation was one of the earliest biological functions associated with STAT proteins, from the anti-viral functions of STAT1, to the polarized T helper cell responses that required STAT4 and STAT6. STATs are also acetylated, methylated, sumoylated, and ubiquitylated, which alters their stability, dimerization, nuclear localization, transcriptional activation function, and association with histone acetyltransferases and histone deacetylases [78]. Importantly, Jak/Stat activation is tightly regulated through the expression of positive (cytokines, receptors, tyrosine kinases) and negative regulators (tyrosine phosphatases, protein inhibitors of activated Stat, suppressor of cytokine signaling [SOCS] proteins) [78].

In this signaling pathway, binding of a cytokines such as IL-4 or IL-12 to their receptors leads to the activation of members of the JAK family of receptor-associated kinases. These kinases subsequently activate, via tyrosine phosphorylation, preexistent cytoplasmic factors termed STATs. Tyrosine phosphorylation allows the STAT proteins to dimerize and translocate to the nucleus, where they mediate changes in gene expression by binding specific DNA elements. The SOCS (suppressors of cytokine signaling) family proteins (in particular SOCS3) are the best understood negative regulators of the JAK-STAT pathway [10, 80-82], and are composed of eight proteins, i.e. SOCS1–7 and SH2 cytokine-inducible protein (CIS) [80-82]. The regulatory function of SOCS proteins is critical to the normal functioning and cessation of the primary cytokine signal and it is achieved at many levels in the intracellular biochemical cascade [80-82]. For example, SOCS1 is thought to inhibit the catalytic activity of JAKs by binding to the activation loop of the catalytic domain through both its kinase inhibitory region (KIR) and SH2 domain. Binding of SOCS1 to JAK kinases therefore blocks further signaling in a negative feedback loop [80-82]. Exercise has been shown to modulate SOCS-JAK-STAT signaling pathways in different cells and tissues including blood, testis, kidney, skeletal muscle, endothelial cells, heart and lungs [14-19, 48]. However, no information is available concerning the potential involvement of SOCS, JAK and STAT signaling in the beneficial effects of aerobic exercise in asthma. We have recently investigated the effects of aerobic exercise on STAT3 expression in the lungs of smoked-exposed animals [14]. We demonstrated that aerobic exercise reduced smoke-induced STAT3 expression and phosphorylation in airway epithelial cells, peribronchial leukocytes, and parenchymal leukocytes. The reduction in STAT3 expression and phosphorylation was accompanied by reduction in smoked-induced inflammatory cytokines (IL-1β, IL-17, TNF-α) and induction of IL-10 levels in BALF and serum in the mice.

Therefore, we hypothesized that the anti-inflammatory effects of aerobic exercise in an experimental model of asthma, would be due to its effects on inflammatory cytokines, involving SOCS-JAK-STAT signaling pathway.

Therefore, we aimed to perform an original study to investigate the potential role of SOCS-JAK-STAT signaling pathway in the effects of aerobic exercise training on airway inflammation, remodeling and hyperresponsiveness in a

model of house dust mite-induced allergic airway inflammation. Of note, this is the first study testing the effects of aerobic exercise in face a real life allergen (house dust mite – *dermatophagoides pteronyssinus*) inducing an asthma phenotype, through a direct contact with the respiratory mucosa, like happens in humans.

MATERIALS AND METHODS

Animals and study design

The experimental protocol was approved by the ethical committee of the Nove de Julho University. All animal care and experimental procedures followed the international recommendations of the Helsinki convention for the use and care of animals.

120 male C57Bl/6 mice (aged 8 weeks and weighing 20g approximately) were maintained under standard conditions with controlled temperature (22°C - 25°C) and relative humidity (50%-60%) on a 12 h light/dark cycle. They were provided with food and water *ad libitum*. The animals were randomly distributed into the following experimental groups (n = 3 x 10 animals in each group): 1. Control (Con - not sensitized and untrained), 2. Exercise (Exe - not sensitized and trained), 3. HDM (HDM - sensitized to HDM and untrained), 4. HDM + Exe (HDM + Exe - sensitized with HDM and trained).

Protocol of chronic allergic lung inflammation

Under anesthesia using ketamine (100 mg/kg) and xylazine (10 mg/kg), HDM groups received *Dermatophagoides pteronyssinus* extract (HDM 100µg/mouse) (Greer Laboratories, Lenoir, NC) diluted in 50µl of phosphate buffered saline (PBS), orotracheally administered, on days 0, 7, 14, 21, 28, 35 e 42 [36].

Physical test and exercise training protocol

On days 14 to 16 mice were placed on the treadmill (Inbramed, Brazil) for 15 min at a speed of 0.5 km/h and a 15% incline for adaptation to avoid stress induction [34,35]. On days 17 and 42 the maximal exercise test was performed as previously described [34,35]. The treadmill training occurred to 50% of maximal exercise capacity reached in the physical exercise test, which correspond to the moderate intensity [34,35]. It has begun on day 18 and was performed over 4 weeks, 60 min per session for five days a week.

Total and differential cell counting in bronchoalveolar lavage fluid (BALF)

The lungs were carefully washed with 1.5 ml of saline (3×0.5 ml) via tracheal cannula. The samples were centrifuged (900 ×g for 7 min at 4°C), and the resulting cell pellet was re-suspended in PBS (1 ml). BALF total cell count were performed under staining using trypan blue using a hematocytometer (Neubauer chamber) [34]. The differential cell count was carried out after cytocentrifuge preparations (Cytospin®, Fanem, Brazil) stained with May–Grünwald–Giemsa solution [34].

Cytokine measurements in BALF

The BALF levels of IL-4 (DY404; assay range: 15.6 - 1,000 pg/mL), IL-5 (DY405; assay range: 31.2 - 2,000 pg/mL), IL-13 (DY413; assay range: 62.5 - 4,000 pg/mL), CXCL1 (DY453; assay range: 15.6 - 1,000 pg/mL), IL-17 (DY421; assay range: 15.6 - 1,000 pg/mL), IL-23 (DY1887; assay range: 39.1 - 2,500 pg/mL), IL-33 (DY3626; assay range: 15.6 - 1,000 pg/mL) and IL-10 (DY417; assay range: 31.2 - 2,000 pg/mL) were determined by ELISA, using R&D Systems Duo Set kits (MN, USA), according to the manufacturer's recommendations. All reads were done in a SpectraMax i3 microplate reader (Molecular Devices, CA, USA).

Airway inflammation and remodeling

The lungs were removed in bloc and perfused and fixed under positive pressure of 20 cmH_2O with 4% paraformaldehyde solution for 24 hours. The lungs were embedded in paraffin and sectioned in 4 µm slices. The staining was performed with hematoxylin and eosin (HE) for quantification of eosinophils, lymphocytes, neutrophils and macrophages in the peribronchial space, with Picrossirius, for quantification of collagen fibers and with Weigert's resorcin–fuchsin with oxidation for quantification of elastic fibers in airways wall [34]. Periodic Schiff acid plus blue alcian was used for quantification of mucus production in airway epithelium [30]. Five airways of each mouse were used for the analysis [30,34]. All images were taken using a camera QColor5 (Olympus, PA, USA) attached to a microscope Olympus BX40 (Olympus, PA, USA), while the image analysis was done using CellSens software (Olympus, PA, USA) [30].

Immunolocalization and quantification of SOCS1, SOCS2, SOCS3, STAT3, STAT5, STAT6 and JAK2

Immunohistochemistry was performed in 4 µm slices, which were incubated overnight at 4°C with the following primary antibodies: anti-SOCS1 (sc-7006; 1:2.000), anti-SOCS2 (sc-7008; 1:2.000), anti-SOCS3 (sc-7010; 1:2.000), anti-JAK2 (sc-278; 1:20.000), anti-STAT3 (sc-482; 1:20.000), anti-STAT5 (sc-835; 1:20.000) and anti-STAT6 (sc-981; 1:20.000) (Santa Cruz Biotechnology, CA, USA). The reaction was followed by incubation with proper secondary antibodies conjugated with biotin-streptavidin-peroxidase and counter-stained with Harris' hematoxylin, as previously described [20,21,26,29,30,34,35]. Since the immunoreaction was observed in airway epithelium and in peribronchial leukocytes, the quantitative analysis of the expression of each protein was done as follow, in five airways of each mouse:

Positive peribronchial leukocytes: the number of positive peribronchial leukocytes in the peribronchial space (area comprehended between airway basal membrane and airway adventitia) was counted, and the results expressed as number of positive cells per square millimeter [30,35].

Positive area of airway epithelium: the total area of airway epithelium was measured, and the positive area of airway epithelium for each protein was quantified. Then the results were expressed as percentage of airway epithelium positive for each protein [30,34].

Evaluation of airway hyperresponsiveness (AHR)

AHR was evaluated in conscious mice using whole body plethysmograph (Buxco Europe, Winchester, UK) to growing doses (Basal, PBS, 6,25 mg/mL, 12,5 mg/mL, 25 mg/mL and 50 mg/mL) of methacholine (MCh), by using the enhanced

pause (Penh), which correspond to the level of airway obstruction [30].

Statistical analysis

All data were analyzed, and the graphs were built using the software GraphPad Prism 5.0 (CA, USA). Since all data presented parametric distribution, statistical analysis was performed by one-way analysis of variance (ANOVA ONE-WAY) and by Student-Newman-Keuls as post-hoc test. P <0.05 was considered significant. All graphs were presented as mean and standard deviation.

RESULTS

Effects of aerobic exercise on physical capacity and on body weight

The results showed that comparing the initial with final physical test in terms of time (minutes) the Control (2.4±2.04 min; p>0.05) and HDM (3.25±3.95 min; p>0.05) groups did not present significant increases in physical capacity. On the other hand, Exercise (13.9±5.27 min; p<0.05) and HDM+Exercise (11.91±3.23 min; p<0.05) presented significant improvements. When the body weight was analyzed (final body weight minus the initial body weight), the results showed that no significant differences were found (p<0.05).

Aerobic exercise reduces pulmonary inflammation

Figure 2 shows that the HDM model of chronic allergic airway inflammation significantly increases the number of total cells (Figure 2A; p<0.05), eosinophils (Figure 2B; p<0.01), neutrophils (Figure 2B; p<0.01) and lymphocytes (Figure 2B; p<0.05) in BALF. On other way, aerobic exercise reduces HDM-induce increases the number of total cells (Figure 2A; p<0.05), eosinophils (Figure 2B; p<0.05), neutrophils (Figure 2B; p<0.05) and lymphocytes (Figure 2B; p<0.05) in BALF.

Complementarily, quantitative histological analysis revealed that HDM model of chronic allergic airway inflammation significantly increases the number of eosinophils (Figure 3A; p<0.001), neutrophils (Figure 3B; p<0.001) and lymphocytes (Figure 3C; p<0.01) in the peribronchial space. Again, aerobic exercise was able to reduce HDM-induce increases the number of eosinophils (Figure 3A; p<0.001), neutrophils (Figure 3B; p<0.001) and lymphocytes (Figure

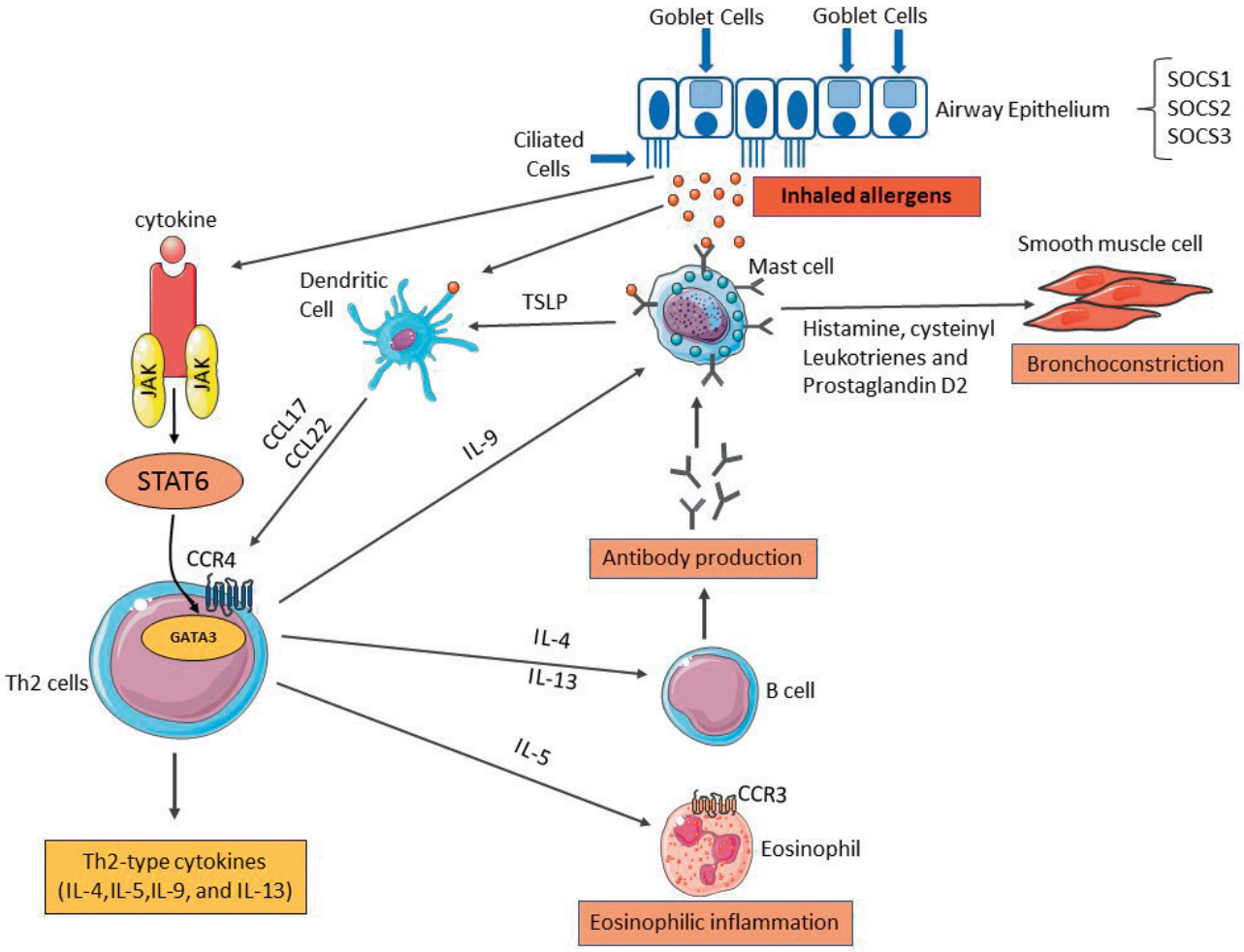


Figure 1. Inflammatory and immune cells involvement in asthma. Inhaled allergens activate lung Dendritic cells and Mast cells to release several chemotactic factors. Activated dendritic cells release the chemokines CCL17 and CCL22, which act on Chemokine-receptor 4 (CCR4) to recruit T helper 2 (TH2) cells. Activation of the transcription factor GATA3 in TH2 cells leads to secretion of the cytokines IL-4 and IL-13 (which stimulate B cells to synthesize IgE), IL 5 (which is necessary for eosinophilic inflammation) and IL 9 (which stimulates mast-cell proliferation). Activated mast cells release several bronchoconstriction mediators including cysteinyl leukotrienes and prostaglandin D2. TSLP: thymic stromal lymphopoietin., CCL17: CC chemokine ligand 17., CCL22: CC chemokine ligand 22., CCR3: CC- chemokine receptor 3., CCR4: CC-chemokine receptor 4., IL-4: interleukin 4, IL-5: interleukin 5, IL-9: interleukin 9., IL-13: interleukin 13., GATA3: GATA binding protein 3.

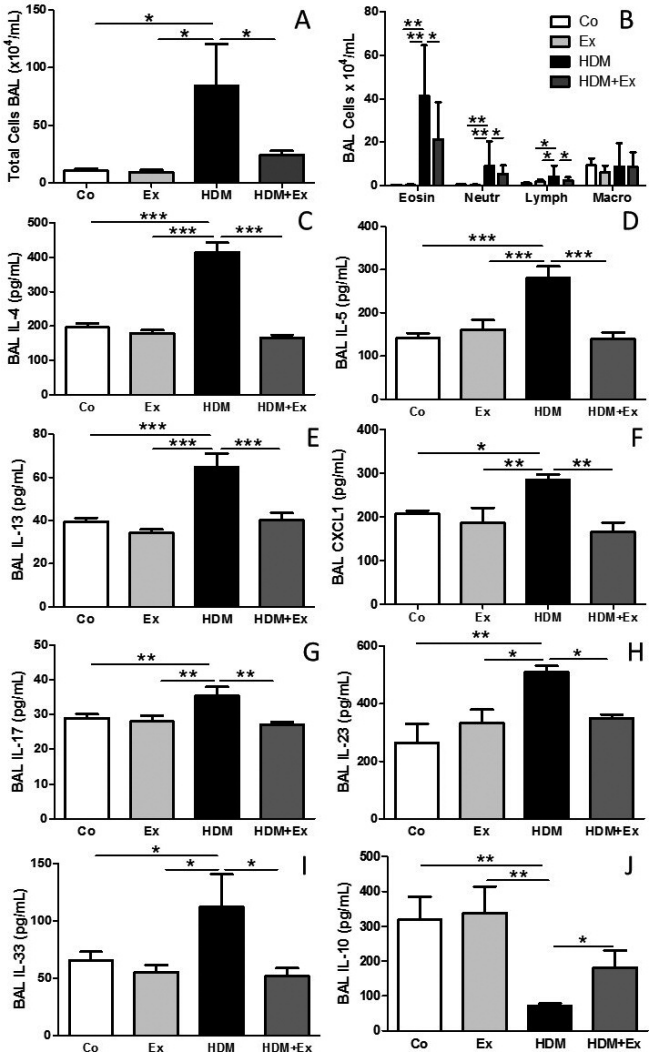


Figure 2. Figure 2 shows the number of total cells in BAL (Figure 2A), eosinophils, neutrophils, lymphocytes and macrophages in BAL (Figure 2B), IL-4 levels in BAL (Figure 2C), IL-5 levels in BAL (Figure 2D), IL-13 levels in BAL (Figure 2E), CXCL1 levels in BAL (Figure 2F), IL-17 levels in BAL (Figure 2G), IL-23 levels in BAL (Figure 2H), IL-33 levels in BAL (Figure 2I) and IL-10 in BAL (Figure 2J). * p<0.05, ** p<0.01 and *** p<0.001.

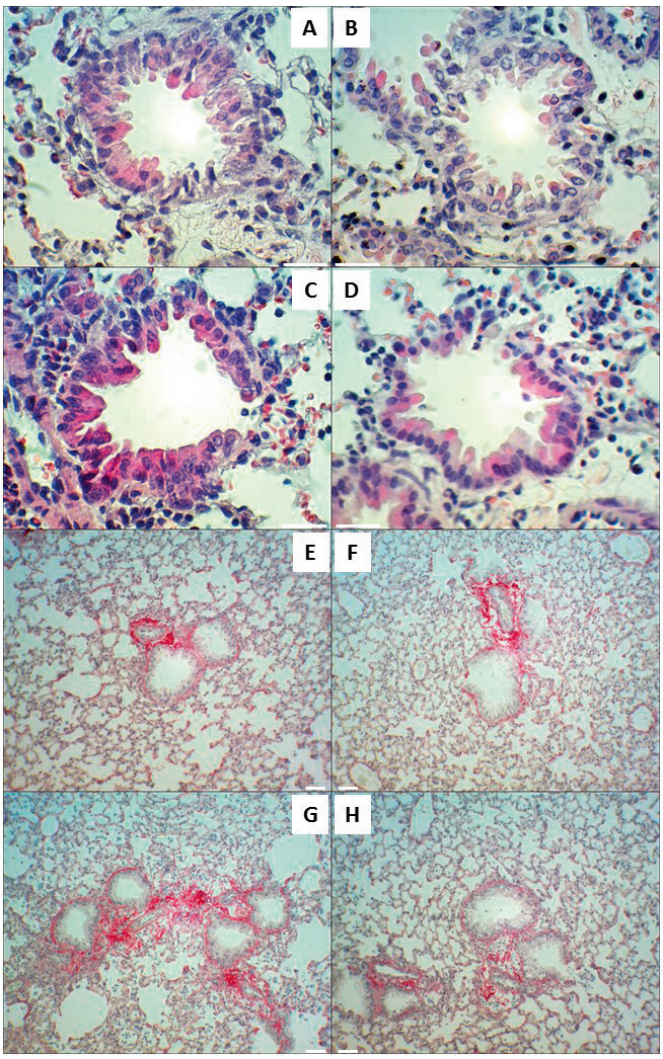


Figure 3. Figure 3 shows the density of eosinophils (Figure 3A), neutrophils (Figure 3A) and lymphocytes (Figure 3C) in airways wall, and of collagen fibers (Figure 3D), elastic fibers (Figure 3E) and mucin (Figure 3F) in the airways. * p<0.05, ** p<0.01 and *** p<0.001.

3C; p<0.001) in the peribronchial space. Figure 4A-D shows representative photomicrographs taken from HE staining, through which the peribronchial inflammation analysis were done.

Aerobic exercise reduces pro-inflammatory cytokines and increases IL-10

Figure 2 shows that the HDM model of chronic allergic airway inflammation significantly increases the BALF levels of Th2 cytokines IL-4 (Figure 2C; p<0.001), IL-5 (Figure 2D; p<0.001), IL-13 (Figure 2E; p<0.001), Th17 cytokine IL-17 (Figure 2G; p<0.01), IL-23 (Figure 2H; p<0.01), IL-33 (Figure 2I; p<0.05) and CXCL1 (Figure 2F; p<0.05), while reduces the levels of IL-10 (Figure 2J; p<0.01). On contrary, aerobic exercise reduces HDM-induce increases in the levels of Th2 cytokines IL-4 (Figure 2C; p<0.001), IL-5 (Figure 2D; p<0.001), IL-13 (Figure 2E; p<0.001), Th17 cytokine IL-17 (Figure 2G; p<0.01), IL-23 (Figure 2H; p<0.05), IL-33 (Figure 2I; p<0.05) and CXCL1 (Figure 2F; p<0.01), while increases the levels of IL-10 (Figure 2J; p<0.05).

Aerobic exercise reduces airway remodeling

Figure 3 shows that the HDM model of chronic allergic airway inflammation significantly increases airway remodeling, notably through accumulation of collagen fibers in airways wall (Figure 3D; p<0.05), elastic fibers in airways wall (Figure 3E; p<0.001), and mucin production by airway epithelium (Figure 3F; p<0.001). Importantly, aerobic exercise was able to reduces HDM-induce increases in airway remodeling, as noted through accumulation of collagen fibers in airways wall (Figure 3D; p<0.05), elastic fibers in airways wall (Figure 3E; p<0.01), and mucin production by airway epithelium (Figure 3F; p<0.01). Figure 4E-G shows representative photomicrographs taken from Picrossirius staining, through which the collagen fibers accumulation in airways wall analysis were done.

Aerobic exercise reduces airway hyperresponsiveness (AHR)

Figure 5 shows that the HDM model of chronic allergic airway inflammation significantly increases AHR, as demon-

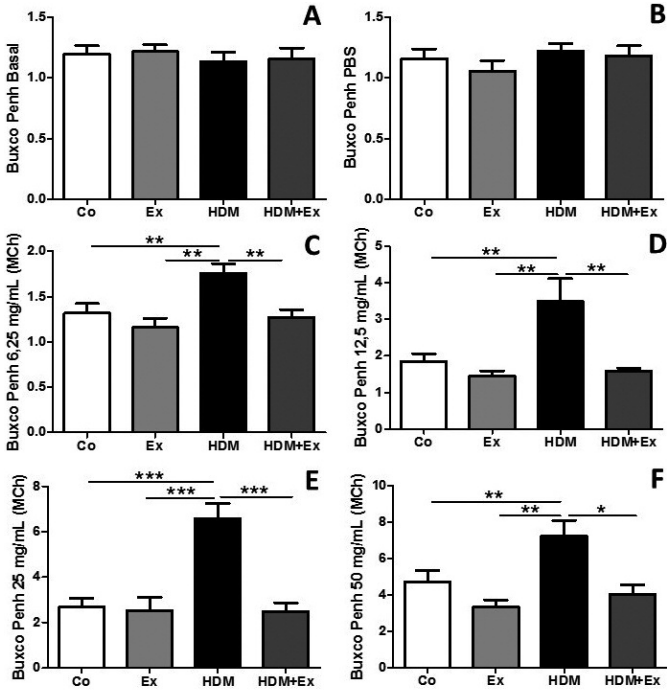


Figure 4. Figure 4 shows representative photomicrographs of quantitative histological analysis, being stained with hematoxylin and eosin for analysis of airway inflammation (Figure 4A; 400x magnification) and with picrosirius for analysis of collagen accumulation in the airways (Figure 4B; 200x magnification). Scale bar are 25 µm and 50 µm, respectively.

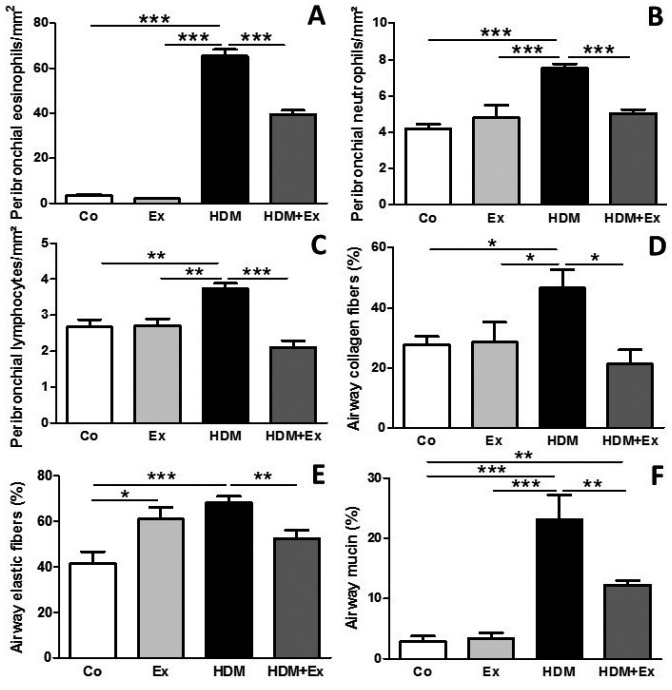


Figure 5. Figure 5 shows airway hyperresponsiveness (AHR) for growing doses of methacholine (Mch), measured using whole body plethysmography. The results are demonstrated as enhanced pause (Penh). * p<0.05, ** p<0.01 and *** p<0.001.

strated through methacholine (MCh) aerosol challenge: 6,25 mg/mL (Figure 5C; p<0.01), 12,5 mg/mL (Figure 5D; p<0.01), 25 mg/mL (Figure 5E; p<0.001) and 50 mg/mL (Figure 5F; p<0.01). Of note, aerobic exercise significantly reduces HDM-induce increases in AHR, as demonstrated through methacholine (MCh) aerosol challenge: 6,25 mg/mL (Figure 5C; p<0.01), 12,5 mg/mL (Figure 5D; p<0.01), 25 mg/mL (Figure 45E; p<0.001) and 50 mg/mL (Figure 5F; p<0.05).

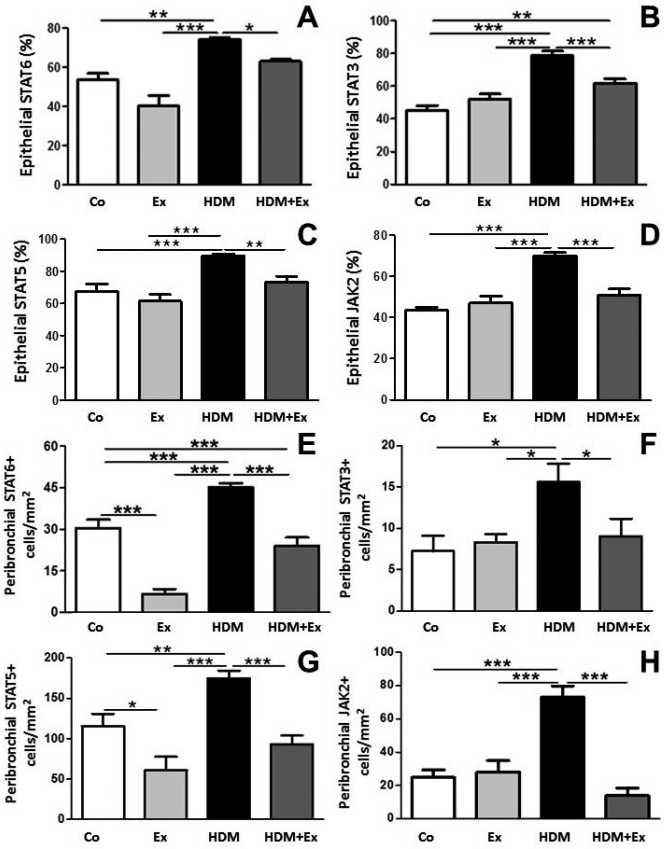


Figure 6. Figure 6 shows the epithelial expression of STAT6 (Figure 6A), STAT3 (Figure 6B), STAT5 (Figure 6C) and JAK2 (Figure 6D) and of STAT6 (Figure 56E), STAT5 (Figure 6F), STAT3 (Figure 6G) and JAK2 (Figure 6H) by peribronchial leukocytes. * p<0.05, ** p<0.01 and *** p<0.001.

Aerobic exercise reduces STAT6, STAT3 and STAT5 and JAK2 expression

Figure 6 shows that the HDM model of chronic allergic airway inflammation significantly increases epithelial expression of STAT6 (Figure 6A; p<0.01), STAT3 (Figure 6B; p<0.001), STAT5 (Figure 6C; p<0.001) and JAK2 (Figure 6D; p<0.001). In addition, HDM model of chronic allergic airway inflammation significantly increases the expression STAT6 (Figure 6E; p<0.001), STAT3 (Figure 6F; p<0.05), STAT5 (Figure 6G; p<0.01) and JAK2 (Figure 6H; p<0.001) by peribronchial leukocytes. Contrarily, aerobic exercise significantly reduces HDM-induce increases in epithelial expression of STAT6 (Figure 6A; p<0.05), STAT3 (Figure 6B; p<0.001), STAT5 (Figure 6C; p<0.01) and JAK2 (Figure 6D; p<0.001). In addition, aerobic exercise also reduced HDM-induce increases the expression STAT6 (Figure 6E; p<0.001), STAT3 (Figure 6F; p<0.05), STAT5 (Figure 6G; p<0.001) and JAK2 (Figure 6H; p<0.001) by peribronchial leukocytes. Figure 7 shows representative photomicrographs taken from STAT6 (Figure 7 A-D) and from STAT3 (Figure 7 E-H) immunostaining.

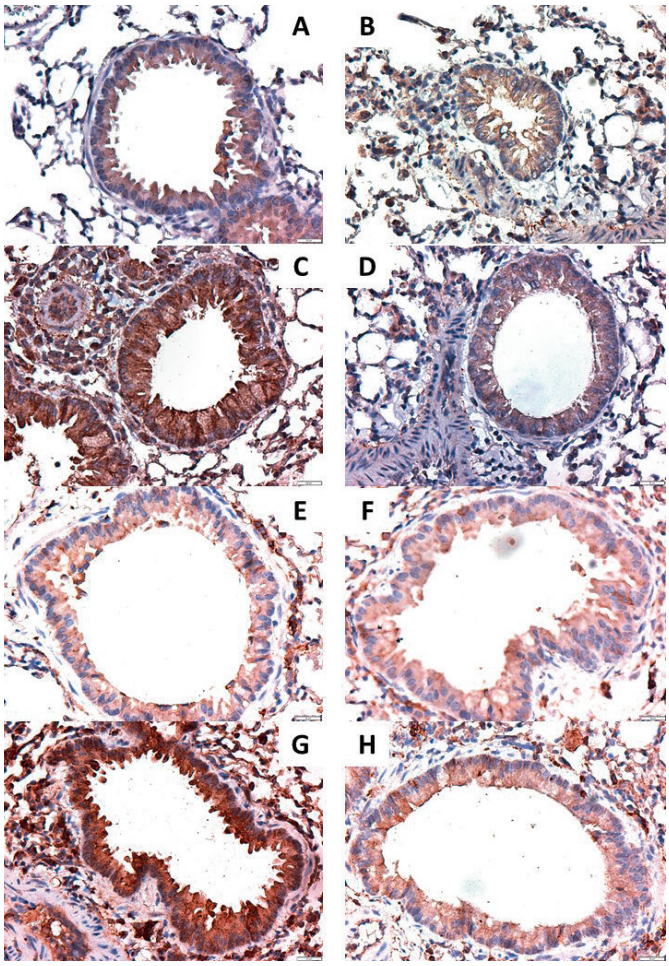


Figure 7. Figure 7 shows representative photomicrographs of quantitative immunohistochemistry analysis of the expression of STAT6 (Figure 7A-D; Co, Ex, HDM and HDM+Ex groups, respectively) and STAT3 (Figure 7E-H; Co, Ex, HDM and HDM+Ex groups, respectively). Images are at 400x magnification. Scale bar are 25 μm.

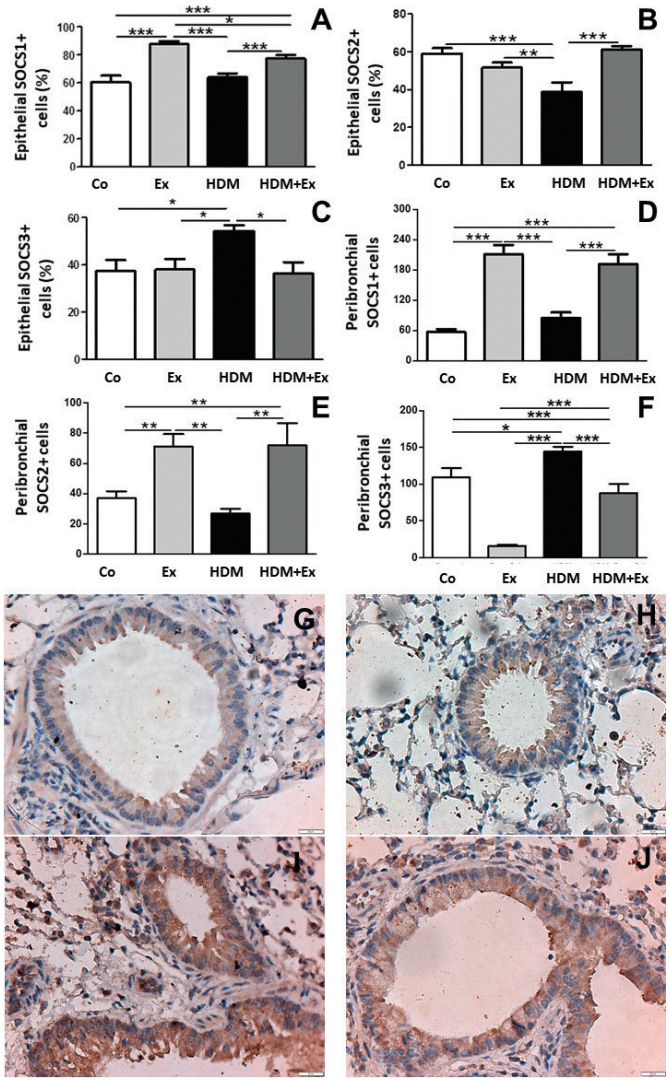


Figure 8. Figure 8 shows the quantitative analysis of the expression of SOCS1 (Figure 8A), SOCS2 (Figure 8B), SOCS3 (Figure 8C) by airway epithelium and of SOCS1 (Figure 8D), SOCS2 (Figure 8E), SOCS3 (Figure 8F) by peribronchial leukocytes. * p<0.05, ** p<0.01 and *** p<0.001. Figure 8G-J shows representative photomicrograph of quantitative immunohistochemistry analysis of the expression of SOCS3, of Co, Ex, HDM and HDM+Ex groups, respectively. Images are at 400x magnification. Scale bar are 25 μm.

Aerobic exercise modulates SOCS1, SOCS2 and SOCS3 expression

Figure 8 shows that the HDM model of chronic allergic airway inflammation significantly does not change epithelial expression of SOCS1 (Figure 8A; p>0.05), but reduces epithelial expression of SOCS2 (Figure 8B; p<0.001), while increases epithelial expression of SOCS3 (Figure 8C; p<0.05). In addition, HDM model of chronic allergic airway inflammation does not change the expression of SOCS1 (Figure 8D; p>0.05) and SOCS2 (Figure 8D; p>0.05), by peribronchial leukocytes, but increases the expression of SOCS3 by peribronchial leukocytes (Figure 8F; p<0.05). Of importance, aerobic exercise in non-sensitized (Ex) and in sensitized (HDM+Ex) mice significantly increases the epithelial expression of SOCS1 (Figure 8A; p<0.001). Aerobic exercise also restores the epithelial expression of SOCS2 (Figure 8B; p<0.001), while reduces HDM-induce epithelial expression of SOCS3 (Figure 8C; p<0.05). Regarding the expression SOCS1 (Figure 8D; p<0.001) and SOCS2 (Figure 8E; p<0.01) by peribronchial leukocytes, aerobic exercise increases their expression in non-sensitized and in sensitized mice. Furthermore, aerobic exercise reduces HDM-induce the expression of SOCS3 by peribronchial leukocytes (Figure 8F; p<0.001). Figure 8 shows representative photomicrographs taken from SOCS3 (Figure 8 G-J) immunostaining.

DISCUSSION

The present study shows for the first time the involvement of SOCS-JAK-STAT signaling on the beneficial effects of regular aerobic exercise at low intensity reducing asthma phenotype, denoted as reduced eosinophilic inflammation, Th2 immune response, airway remodeling and AHR. In addition, this is the first study performing a complete description of several SOCS-JAK-STAT proteins in a model of HDM-induced asthma.

Asthmatic airway inflammation is characterized by accumulation of several cell types in airways wall, including mast cells, dendritic cells, Th2 lymphocytes and eosinophils [2,3]. However, eosinophilic inflammation is considered a hallmark of asthmatic airway inflammation and its levels is correlated

to asthma severity and risk of exacerbations [37]. The literature already has shown that aerobic exercise can reduces eosinophilic inflammation in asthmatics patients [38] and in experimental models of asthma induced by ovalbumin [30-35], but this is the first study showing that aerobic exercise can reduces eosinophilic inflammation in an experimental model of HDM-induced asthma phenotype. Thus, using a more physiological model of asthma using HDM, in which sensitization occurs through a direct contact activating with airways mucosa, i.e. airway epithelium [36,39], reinforce the importance of aerobic exercise, the main component of a program of pulmonary rehabilitation, in the control of eosinophilic inflammation for asthma.

Th2 cytokines, such as IL-4, IL-5 and IL-13 present an essential role in asthma pathogenesis and progression [4,8,30-36,39]. Previous literature shows that aerobic exercise inhibits accumulation of Th2 in BALF in ovalbumin models of asthma [30-35]. Such inhibitory effects of AE are of importance, considering that Th2 cytokines are also involved not only in the asthmatic inflammatory response, but also in the remodeling and in the AHR [4,8,10]. Furthermore, IL-17 a Th17- and epithelial-derived cytokine is thought to be involved in the proliferation and activation of fibroblast, airway smooth muscle and also in IL-8/CXCL-1 chemokine release, contributing to impairment of airway remodeling, AHR and to the inflammatory response, attracting neutrophils to the airways [5,40]. In addition, the main inflammatory consequence of IL-17 inducing the release of CXCL-1 attracting neutrophils to the airways, was observed in the present study, since the HDM model used in this study also induced increases in the levels of CXCL-1 and in the number of neutrophils in the lungs. In contrast, the present study showed for the first time that AE was able to inhibits HDM-induced IL-17 accumulation in the lungs, as demonstrated by reduced levels of IL-17 in BALF, effects that were followed by reduced levels of BALF CXLC-1 and by reduced numbers of neutrophils in BAL. Such effects of AE suggest that perhaps, AE can inhibit not only eosinophilic asthma, but also difficult to treat asthma, which is characterized by increased number of neutrophils in the airways [5,40].

IL-23 has been recently described as an important cytokine involved in asthma pathophysiology, mainly produced by dendritic cells, macrophages and airway epithelial cells [5,41]. It is involved primarily in the control of IL-17 synthesis, but also with the sensitization process, beyond to contribute to recruitment of both eosinophils and neutrophils to the airways [5,41]. IL-23 also seems to induce IL-33 synthesis and release, a cytokine involved in several aspects of asthma pathogenesis and maintenance, as in inflammation, remodeling and even in AHR [42]. IL-33 drives Th2 cells recruitment, activation, polarization through NF-kB activation, beyond to increase dendritic cells maturation and activation, which are cell types presenting a key role for asthma development [42]. Here, we demonstrated for the first time that AE was able to reduce IL-17, IL-23 and IL-33 in BALF of HDM-stimulated mice, demonstrating an extensive anti-inflammatory role of AE in the context of asthma. Of note, part of these anti-inflammatory effects can be attributed to AE-induced IL-10 release, which is an anti-inflammatory cytokine, that positively contributes to the anti-inflammatory effects of AE, as previously demonstrated [14,20,21,23,24,26,27].

Beyond exacerbated airway inflammation, airway remodeling is a hallmark of asthma, which is characterized by increased sub-epithelial deposition of extracellular matrix proteins (i.e. collagen and elastic fibers, proteoglycans and laminins), hypertrophy and hyperplasia of airway smooth muscle and epithelium and basal membrane thickness [6,7]. These structural changes in the airways remains as main challenge for treatment of asthma and are closely related to severity of the disease, to airway obstruction, breathlessness and to AHR [6,7]. In the present study, it was observed for the first time in a model of HDM-induced airway remodeling that AE reduced collagen and elastic fibers accumulation in the airways wall as well as reduced mucus production by airway epithelium, reinforcing the anti-fibrotic effects of AE, which has been already demonstrated in models of ovalbumin-induced asthma [30-35], COPD [14,20-22] and pulmonary fibrosis [23,24]. In addition, the importance of the anti-fibrotic effects of AE, which was observed in the present study, could be, at least in part correlated to the inhibitory effects of AE on AHR, which occurs in response to several factors, such as airway inflammation and remodeling [7]. Also, this inhibitory effect on AHR is particularly important, displaying that the anti-inflammatory and anti-fibrotic effects of AE result in improvement of functional response of the lungs.

Cytokine signaling depends of activation of intracellular molecules, such JAK and STATs [8-12], while JAK and STATs activation can be inhibited by SOCS proteins [8-12]. However, the literature is not unanimous concerning the inhibitory effects of SOCS proteins, since SOCS1, for instance, is upregulated in nasal epithelial cells of asthmatics and correlates with asthma severity [8]. On the other hand, it has been demonstrated that absence of SOCS1 resulted in increased asthma phenotype in a model of ovalbumin-induced asthma [43]. In the present study, which was performed using a HDM model of asthma, no changes in the expression of SOCS1 by peribronchial leukocytes or by airway epithelium were found. However, AE resulted in increased expression of SOCS1 by peribronchial leukocytes and by airway epithelium in non-sensitized and in sensitized mice groups, effect that can be involved in the inhibitory effects of AE on asthma phenotype, since that it has been demonstrated that SOCS1 suppress IL-13-dependent STAT6 activation, which constitute a central pathway for asthma development [44]. This effect of AE increasing SOCS1 expression can be reinforced, since AE not only reduced HDM-induced asthma phenotype, but also reduced the expression of STAT6, STAT5, STAT3 and JAK2 by peribronchial leukocytes and by airway epithelium. However, a direct causal effects cannot be definitively proved in the present study.

Concerning SOCS2, it was observed that HDM administration significantly reduced epithelial expression of SOCS2, while only a slight reduction in SOCS2 expression by peribronchial leukocytes was observed. In addition, these effects were followed by enhanced expression of STAT6, STAT5, STAT3 and JAK2 by peribronchial leukocytes and by airway epithelium. These findings are in partial agreement with a study from Knosp et al 2011, where the authors demonstrated exacerbated asthmatic phenotype and increased activation of STAT6 and STAT5 in SOCS2 ko mice [10]. On the other hand, AE was able to restore epithelial SOCS2 expression and to increase significantly SOCS2 expression by peribronchial

leukocytes, suggesting a possible mechanism underlying the effects of AE on asthma. However, whether the anti-asthmatic effects of AE are dependent of SOCS2 remain to be further investigated.

Increased expression of SOCS3 have been described in T-cells of asthmatic patients correlating to onset and maintenance of Th2 immune response and increased IgE levels [8]. In addition, another study showed that SOCS3 expression is increased in eosinophils of asthmatic patients and is functionally involved in eosinophil migration, adhesion and degranulation involving STAT3 activation [45]. Furthermore, it has been demonstrated that ovalbumin model of asthma results in increased expression of SOCS3 in the lungs, and that silencing of SOCS3 abrogates asthma phenotype [46]. In line with the current literature, the present study found that HDM increases SOCS3 and also STAT3 expression by airway epithelium and by peribronchial leukocytes. Such effects were significantly inhibited by AE, reinforcing the potential immunomodulatory role of AE on asthma involving SOCS-JAK-STAT signaling.

In conclusion, aerobic exercise inhibits house dust mite induce asthma phenotype, involving the modulation of SOCS-JAK-STAT signaling in airway epithelium and in peribronchial leukocytes. In addition, these experimental results point out a possible immunological and molecular mechanism underlying the beneficial effects of aerobic exercise on asthma phenotype, which should be urgently investigated in a clinical study in asthmatic individuals.

ACKNOWLEDGEMENTS

This study was financially supported by São Paulo Research Foundation (FAPESP), grants 2012/15165-2, 2012/16498-5 and 2015/50010-0. ARAO holds a MSc fellowship from FAPESP (2014/07500-1). JCJAJ holds a MSc fellowship from FAPESP (2014/12755-9). MCOJ holds a PhD fellowship from FAPESP (2014/14504-8). RWAC holds a MSc fellowship from FAPESP (2012/21519-1). NCRO holds a MSc fellowship from FAPESP (2013/24888-0). The results and opinions presented in this study does not reflect the vision and opinion from FAPESP.

REFERENCES

1. von Mutius E. Gene-environment interactions in asthma. J Allergy Clin Immunol (2009) 123(1):3–11. doi:10.1016/j.jaci.2008.10.046
2. Global Initiative for Asthma (GINA).
3. Liu MC, Xiao HQ, Breslin LM, Bochner BS, Schroeder JT. Enhanced antigen presenting and T cell functions during late-phase allergic responses in the lung. Clin Exp Allergy. 2017 Nov 3. doi: 10.1111/cea.13054. [Epub ahead of print].
4. Nath P, Leung SY, Williams AS, et al. Complete inhibition of allergic airway inflammation and remodelling in quadruple IL-4/5/9/13-/- mice. Clin Exp Allergy 2007; 37: 1427–1435.
5. Gupta RK, Gupta K, Dwivedi PD. Pathophysiology of IL-33 and IL-17 in allergic disorders. Cytokine Growth Factor Rev 2017 Dec,38:22-36. doi: 10.1016/j.cytogfr.2017.09.005. Epub 2017 Nov 11.
6. Samitas K, Carter A, Kariyawasam HH, Xanthou G. Upper and lower airway remodelling mechanisms in asthma, allergic rhinitis and chronic rhinosinusitis: The one airway concept revisited. Allergy. 2017 Dec 1. doi: 10.1111/all.13373. [Epub ahead of print] Review.
7. Wang KC, Le Cras TD, Larcombe AN, Zosky R, Elliot JG, James AL, Noble PB. Independent and combined effects of airway remodelling and allergy on airway responsiveness. Clin Sci (Lond). 2017 Dec 21. pii: CS20171386. doi: 10.1042/CS20171386. [Epub ahead of print].
8. Gras D, Chanez P. New sociology for better understanding severe eosinophilic asthma: introducing the SOCS family. Eur Respir J. 2016 Sep;48(3):608-10. doi: 10.1183/13993003.01240-2016.
9. Liu Y, Zhang H, Ni R, Jia WQ, Wang YY. IL-4R suppresses airway inflammation in bronchial asthma by inhibiting the IL-4/STAT6 pathway. Pulm Pharmacol Ther. 2017 Apr;43:32-38. doi: 10.1016/j.pupt.2017.01.006. Epub 2017 Jan 16.
10. Knosp CA, Carroll HP, Elliott J, Saunders SP, Nel HJ, Amu S, Pratt JC, Spence S, Doran E, Cooke N, Jackson R, Swift J, Fitzgerald DC, Heaney LG, Fallon PG, Kissenpfennig A, Johnston JA. SOCS2 regulates T helper type 2 differentiation and the generation of type 2 allergic responses. J Exp Med. 2011 Jul 4;208(7):1523-31. doi: 10.1084/jem.20101167. Epub 2011 Jun 6.
11. McCormick SM, Gowda N, Fang JX, Heller NM. Suppressor of Cytokine Signaling (SOCS)1 Regulates Interleukin-4 (IL-4)-activated Insulin Receptor Substrate (IRS)-2 Tyrosine Phosphorylation in Monocytes and Macrophages via the Proteasome. J Biol Chem. 2016 Sep 23;291(39):20574-87. doi: 10.1074/jbc.M116.746164. Epub 2016 Aug 9.
12. Aguilar-Pimentel A, Graessel A, Alessandrini F, Fuchs H, Gailus-Durner V, Hrabě de Angelis M, Russkamp D, Chaker A, Ollert M, Blank S, Gutermuth J, Schmidt-Weber CB. Improved efficacy of allergen-specific immunotherapy by JAK inhibition in a murine model of allergic asthma. PLoS One. 2017 Jun 1;12(6):e0178563. doi: 10.1371/journal.pone.0178563. eCollection 2017
13. Vogiatzis I, Rochester CL, Spruit MA, Troosters T, Clini EM; American Thoracic Society/European Respiratory Society Task Force on Policy in Pulmonary Rehabilitation. Increasing implementation and delivery of pulmonary rehabilitation: key messages from the new ATS/ERS policy statement. Eur Respir J. 2016 May;47(5):1336-41. doi: 10.1183/13993003.02151-2015.
14. Brandão-Rangel MAR, Bachi ALL, Oliveira-Junior MC, Abbasi A, Silva-Renno A, Britto AA, Oliveira APL, Toledo-Arruda AC, Belvisi MG, Vieira RP. Exercise Inhibits the Effects of Smoke-Induced COPD Involving Modulation of STAT-3. Oxid Med Cell Longev 2017 (2017):Article ID 6572714, 13 pages. https://doi.org/10.1155/2017/6572714.
15. Spangenburg EE, Brown DA, Johnson MS, Moore RL. Exercise increases SOCS-3 expression in rat skeletal muscle: potential relationship to IL-6 expression. J Physiol. 2006 May 1;572(Pt 3):839-48.
16. Trenerry MK, Carey KA, Ward AC, Farnfield MM, Cameron-Smith D. Exercise-induced activation of STAT3 signaling is increased with age. Rejuvenation Res. 2008 Aug;11(4):717-24. doi: 10.1089/rej.2007.0643.

17. Yi X, Gao H, Chen D, Tang D, Huang W, Li T, Ma T, Chang B. Effects of obesity and exercise on testicular leptin signal transduction and testosterone biosynthesis in male mice. Am J Physiol Regul Integr Comp Physiol. 2017 Apr 1;312(4):R501-R510. doi: 10.1152/ajpregu.00405.2016.
18. Chen KC, Hsieh CL, Peng CC, Peng RY. Exercise rescued chronic kidney disease by attenuating cardiac hypertrophy through the cardiotrophin-1 -> LIFR/gp 130 -> JAK/STAT3 pathway. Eur J Prev Cardiol. 2014 Apr;21(4):507-20. doi: 10.1177/2047487312462827.
19. Xia WH, Li J, Su C, Yang Z, Chen L, Wu F, Zhang YY, Yu BB, Qiu YX, Wang SM, Tao J. Physical exercise attenuates age-associated reduction in endothelium-reparative capacity of endothelial progenitor cells by increasing CXCR4/JAK-2 signaling in healthy men. Aging Cell. 2012 Feb;11(1):111-9. doi: 10.1111/j.1474-9726.2011.00758.x.
20. Toledo AC, Magalhaes RM, Hizume DC, Vieira RP, Biselli PJ, Moriya HT, Mauad T, Lopes FD, Martins MA. Aerobic exercise attenuates pulmonary injury induced by exposure to cigarette smoke. Eur Respir J. 2012 Feb;39(2):254-64. doi: 10.1183/09031936.00003411.
21. Toledo-Arruda AC, Vieira RP, Guarnier FA, Suehiro CL, Caleman-Neto A, Olivo CR, Arantes PMM, Almeida FM, Lopes FDTQS, Ramos EMC, Cecchini R, Lin CJ, Martins MA. Time-course effects of aerobic physical training in the prevention of cigarette smoke-induced COPD. J Appl Physiol (1985). 2017 Sep 1;123(3):674-683. doi: 10.1152/japplphysiol.00819.2016.
22. do Nascimento ES, Sampaio LM, Peixoto-Souza FS, Dias FD, Gomes EL, Greiffo FR, Ligeiro de Oliveira AP, Stirbulov R, Vieira RP, Costa D. Home-based pulmonary rehabilitation improves clinical features and systemic inflammation in chronic obstructive pulmonary disease patients. Int J Chron Obstruct Pulmon Dis. 2015 Mar 23;10:645-53. doi: 10.2147/COPD.S76216.
23. Pereira PR, Oliveira-Junior MC, Mackenzie B, Chiovatto JE, Matos Y, Greiffo FR, Rigonato-Oliveira NC, Brugemman TR, Delle H, Idzko M, Albertini R, Ligeiro Oliveira AP, Damaceno-Rodrigues NR, Caldini EG, Fernandez IE, Castro-Faria-Neto HC, Dolhnikoff M, Eickelberg O, Vieira RP. Exercise Reduces Lung Fibrosis Involving Serotonin/Akt Signaling. Med Sci Sports Exerc. 2016 Jul;48(7):1276-84. doi: 10.1249/MSS.0000000000000907.
24. Andrade-Sousa AS, Rogério Pereira P, MacKenzie B, Oliveira-Junior MC, Assumpção-Neto E, Brandão-Rangel MA, Damaceno-Rodrigues NR, Garcia Caldini E, Velosa AP, Teodoro WR, Ligeiro de Oliveira AP, Dolhnikoff M, Eickelberg O, Vieira RP. Aerobic Exercise Attenuated Bleomycin-Induced Lung Fibrosis in Th2-Dominant Mice. PLoS One. 2016 Sep 27;11(9):e0163420. doi: 10.1371/journal.pone.0163420. eCollection 2016.
25. Ramos DS, Olivo CR, Quirino Santos Lopes FD, Toledo AC, Martins MA, Lazo Osório RA, Dolhnikoff M, Ribeiro W, Vieira RP. Low-intensity swimming training partially inhibits lipopolysaccharide-induced acute lung injury. Med Sci Sports Exerc. 2010 Jan;42(1):113-9. doi: 10.1249/MSS.0b013e3181ad1c72.
26. Reis Gonçalves CT, Reis Gonçalves CG, de Almeida FM, Lopes FD, dos Santos Durão AC, dos Santos FA, da Silva LF, Marcourakis T, Castro-Faria-Neto HC, Vieira RP, Dolhnikoff M. Protective effects of aerobic exercise on acute lung injury induced by LPS in mice. Crit Care. 2012 Oct 18;16(5):R199. doi: 10.1186/cc11807.
27. Rigonato-Oliveira NC, Mackenzie B, Bachi ALL, Oliveira-Junior MC, Santos-Dias A, Andrade-Sousa A, Delle H, Assumpcao-Neto E, Damaceno-Rodrigues NR, Dulley LH, Abenetti MA, Malfitano C, Angelis K, Albertini R, Oliveira APL, Abbasi A, Northoff H, Vieira RP. Aerobic exercise inhibits acute lung injury: from mouse to human evidence. Exe Immunol Rev 2017;24:36-44.
28. Silva-Renno A, Baldivia GC, Oliveira-Junior MC, Brandao-Rangel MAR, El-Mafarjeh E, Dolhnikoff M, Mauad T, Britto JM, Saldiva PHN, Oliveira LVF, Ligeiro-Oliveira AP, Graudenz GS, Vieira RP. Exercise Performed Concomitantly with Particulate Matter Exposure Inhibits Lung Injury. Int J Sports Med. 2017 Nov 21. doi: 10.1055/s-0043-121147. [Epub ahead of print]
29. Vieira RP, Toledo AC, Silva LB, Almeida FM, Damaceno-Rodrigues NR, Caldini EG, Santos AB, Rivero DH, Hizume DC, Lopes FD, Olivo CR, Castro-Faria-Neto HC, Martins MA, Saldiva PH, Dolhnikoff M. Anti-inflammatory effects of aerobic exercise in mice exposed to air pollution. Med Sci Sports Exerc. 2012 Jul;44(7):1227-34. doi: 10.1249/MSS.0b013e31824b2877.
30. Alberca-Custódio RW, Greiffo FR, MacKenzie B, Oliveira-Junior MC, Andrade-Sousa AS, Graudenz GS, Santos AB, Damaceno-Rodrigues NR, Castro-Faria-Neto HC, Arantes-Costa FM, Martins Mde A, Abbasi A, Lin CJ, Idzko M, Ligeiro Oliveira AP, Northoff H, Vieira RP. Aerobic Exercise Reduces Asthma Phenotype by Modulation of the Leukotriene Pathway. Front Immunol. 2016 Jun 14;7:237. doi: 10.3389/fimmu.2016.00237. eCollection 2016.
31. Mackenzie B, Andrade-Sousa AS, Oliveira-Junior MC, Assumpção-Neto E, Brandão-Rangel MA, Silva-Renno A, Santos-Dias A, Cicko S, Grimm M, Müller T, Oliveira AP, Martins MA, Idzko M, Vieira RP. Dendritic Cells Are Involved in the Effects of Exercise in a Model of Asthma. Med Sci Sports Exerc. 2016 Aug;48(8):1459-67. doi: 10.1249/MSS.0000000000000927.
32. Camargo Hizume-Kunzler D, Greiffo FR, Fortkamp B, Ribeiro Freitas G, Keller Nascimento J, Regina Bruggemann T, Melo Avila L, Perini A, Bobinski F, Duarte Silva M, Rocha Lapa F, Paula Vieira R, Vargas Horewicz V, Soares Dos Santos AR, Cattelan Bonorino K. Aerobic Exercise Decreases Lung Inflammation by IgE Decrement in an OVA Mice Model. Int J Sports Med. 2017 Jun;38(6):473-480. doi: 10.1055/s-0042-121638.
33. Brüggemann TR, Ávila LC, Fortkamp B, Greiffo FR, Bobinski F, Mazzardo-Martins L, Martins DF, Duarte MM, Dafre A, Santos AR, Silva MD, Souza LF, Vieira RP, Hizume-Kunzler DC. Effects of Swimming on the Inflammatory and Redox Response in a Model of Allergic Asthma. Int J Sports Med. 2015 Jun;36(7):579-84. doi: 10.1055/s-0034-1395588.
34. Vieira RP, Toledo AC, Ferreira SC, Santos AB, Medeiros MC, Hage M, Mauad T, Martins Mde A, Dolhnikoff M, Carvalho CR. Airway epithelium mediates the anti-inflammatory effects of exercise on asthma. Respir Physiol Neurobiol. 2011 Mar 15;175(3):383-9. doi: 10.1016/j.resp.2011.01.002.
35. Vieira RP, Claudino RC, Duarte AC, Santos AB, Perini A, Faria Neto HC, Mauad T, Martins MA, Dolhnikoff M, Carvalho CR. Aerobic exercise decreases chronic allergic lung inflammation and airway remodeling in mice. Am J Respir Crit Care Med. 2007 Nov 1;176(9):871-7.

36. Müller T, Grimm M, De Vieira RP, Cicko S, Dürk T, Sorichter S, Zissel G, Idzko M. Local administration of uridine suppresses the cardinal features of asthmatic airway inflammation. Clin Exp Allergy 2010 Oct;40(10):1552-60. doi: 10.1111/j.1365-2222.2010.03518.x.
37. Mendes FA, Almeida FM, Cukier A, Stelmach R, Jacob-Filho W, Martins MA, Carvalho CR. Effects of aerobic training on airway inflammation in asthmatic patients. Med Sci Sports Exerc. 2011 Feb;43(2):197-203. doi: 10.1249/MSS.0b013e3181ed0ea3.
38. Gunsoy NB, Cockle SM, Yancey SW, Keene ON, Bradford ES, Albers FC, Pavord ID. Evaluation of Potential Continuation Rules for Mepolizumab Treatment of Severe Eosinophilic Asthma. J Allergy Clin Immunol Pract. 2017 Dec 16. pii: S2213-2198(17)30914-5. doi: 10.1016/j.jaip.2017.11.026. [Epub ahead of print].
39. Vieira RP, Müller T, Grimm M, von Gernler V, Vetter B, Dürk T, Cicko S, Ayata CK, Sorichter S, Robaye B, Zeiser R, Ferrari D, Kirschbaum A, Zissel G, Virchow JC, Boeynaems JM, Idzko M. Purinergic receptor type 6 contributes to airway inflammation and remodeling in experimental allergic airway inflammation. Am J Respir Crit Care Med. 2011 Jul 15;184(2):215-23. doi: 10.1164/rccm.201011-1762OC.
40. Chesné J, Braza F, Mahay G, Brouard S, Aronica M, Magnan A. IL-17 in severe asthma. Where do we stand? Am J Respir Crit Care Med. 2014 Nov 15;190(10):1094-101. doi: 10.1164/rccm.201405-0859PP.
41. Lee HS, Park DE, Lee JW, Chang Y, Kim HY, Song WJ, Kang HR, Park HW, Chang YS, Cho SH. IL-23 secreted by bronchial epithelial cells contributes to allergic sensitization in asthma model: role of IL-23 secreted by bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol. 2017 Jan 1;312(1):L13-L21. doi: 10.1152/ajplung.00114.2016.
42. Borish L, Steinke JW. Interleukin-33 in asthma: how big of a role does it play? Curr Allergy Asthma Rep. 2011 Feb;11(1):7-11. doi: 10.1007/s11882-010-0153-8.
43. Lee C, Kolesnik TB, Caminschi I, Chakravorty A, Carter W, Alexander WS, Jones J, Anderson GP, Nicholson SE. Suppressor of cytokine signalling 1 (SOCS1) is a physiological regulator of the asthma response. Clin Exp Allergy. 2009 Jun;39(6):897-907. doi: 10.1111/j.1365-2222.2009.03217.x.
44. Fukuyama S, Nakano T, Matsumoto T, Oliver BG, Burgess JK, Moriwaki A, Tanaka K, Kubo M, Hoshino T, Tanaka H, McKenzie AN, Matsumoto K, Aizawa H, Nakanishi Y, Yoshimura A, Black JL, Inoue H. Pulmonary suppressor of cytokine signaling-1 induced by IL-13 regulates allergic asthma phenotype. Am J Respir Crit Care Med. 2009Jun 1;179(11):992-8. doi: 10.1164/rccm.200806-992OC.
45. Zafra MP, Cañas JA, Mazzeo C, Gámez C, Sanz V, Fernández-Nieto M, Quirce S, Barranco P, Ruiz-Hornillos J, Sastre J, del Pozo V. SOCS3 silencing attenuates eosinophil functions in asthma patients. Int J Mol Sci. 2015 Mar 10;16(3):5434-51. doi: 10.3390/ijms16035434.
46. Zafra MP, Mazzeo C, Gámez C, Rodriguez Marco A, de Zulueta A, Sanz V, Bilbao I, Ruiz-Cabello J, Zubeldia JM, del Pozo V. Gene silencing of SOCS3 by siRNA intranasal delivery inhibits asthma phenotype in mice. PLoS One. 2014 Mar 17;9(3):e91996. doi: 10.1371/journal.pone.0091996.
47. Candemir I, Ergun P, Kaymaz D. Efficacy of a multidisciplinary pulmonary rehabilitation outpatient program on exacerbations in overweight and obese patients with asthma. Wien Klin Wochenschr. 2017 Oct;129 (19-20):655-664.
48. Abbasi A, Hauth M, Walter M, Hudemann J, Wank V, Niess AM, Northoff H. Exhaustive exercise modifies different gene expression profiles and pathways in LPS-stimulated and unstimulated whole blood cultures. Brain Behav Immun. 2014 Jul;39:130-41.
49. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. Nat Rev Immunol. 2008 Mar;8(3):183-92. doi: 10.1038/nri2254. Epub 2008 Feb 15. Review.
50. Barnes PJ. Cellular and molecular mechanisms of asthma and COPD. Clin Sci (Lond). 2017 Jul 1;131(13):1541-1558
51. Wenzel, SE. Emergence of biomolecular pathways to define novel asthma phenotypes. Type-2 immunity and beyond. Am. J. Respir. Cell Mol. Biol. (2016) 55, 1–4
52. Al-Ramli, W., Prefontaine, D., Chouiali, F., Martin, J.G., Olivenstein, R., Lemiere, C. et al. T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. J. Allergy Clin. Immunol. 2009 123, 1185–1187
53. Halwani, R., Al-Muhsen, S. and Hamid, Q. T helper 17 cells in airway diseases: from laboratory bench to bedside. Chest 2013 143, 494–501
54. Parsons JP, Mastronarde JG. Exercise-induced bronchoconstriction in athletes. Chest. 2005 Dec;128(6):3966-74
55. Abbasi A, Vieira RP, Northoff H. Letter to the editor: the evidence of exercise-induced bronchoconstriction in endurance runners; genetic basis and gender differences. Exerc Immunol Rev. 2015;21:186-8
56. Weiler JM, Bonini S, Coifman R, et al. American Academy of Allergy, Asthma & Immunology work group report: Exercise-induced asthma. J Allergy Clin Immunol 2007;119(6):1349-58
57. Dogra S, Kuk JL, Baker J, Jamnik V. Exercise is associated with improved asthma control in adults. Eur Respir J. 2011 Feb;37(2):318-23
58. Mancuso CA, Choi TN, Westermann H, Wenderoth S, Wells MT, Charlson ME. Improvement in asthma quality of life in patients enrolled in a prospective study to increase lifestyle physical activity. J Asthma. 2013 Feb;50(1):103-7
59. Fanelli A, Cabral AL, Neder JA, Martins MA, Carvalho CR. Exercise training on disease control and quality of life in asthmatic children. Med Sci Sports Exerc. 2007 Sep;39(9):1474-80
60. Schultz K, Seidl H, Jelusic D, Wagner R, Wittmann M, Faller H, Nowak D, Schuler M. Effectiveness of pulmonary rehabilitation for patients with asthma: study protocol of a randomized controlled trial (EPRA). BMC Pulm Med. 2017 Mar 9;17(1):49
61. Neder JA, Nery LE, Silva AC, Cabral AL, Fernandes AL. Short-term effects of aerobic training in the clinical management of moderate to severe asthma in children. Thorax. 1999 Mar;54(3):202-6
62. Pastva A, Estell K, Schoeb TR, Atkinson TP, Schwiebert LM. Aerobic exercise attenuates airway inflammatory responses in a mouse model of atopic asthma. J Immunol. 2004 Apr 1;172(7):4520-6
63. Vieira RP, Claudino RC, Duarte AC, Santos AB, Perini A, Faria Neto HC, Mauad T, Martins MA, Dolhnikoff M, Carvalho CR. Aerobic exercise decreases chronic allergic lung inflammation and airway remodeling in mice. Am J Respir Crit Care Med. 2007 Nov 1;176(9):871-7
64. Alberca-Custódio RW, Greiffo FR, MacKenzie B, Oliveira-Junior MC, Andrade-Sousa AS, Graudenz GS, Santos AB, Damaceno-Rodrigues NR, Castro-Faria-Neto HC, Arantes-

Costa FM, Martins Mde A, Abbasi A, Lin CJ, Idzko M, Ligeiro Oliveira AP, Northoff H, Vieira RP. Aerobic Exercise Reduces Asthma Phenotype by Modulation of the Leukotriene Pathway. Front Immunol. 2016 Jun 14;7:237

65. Barnes PJ. Anti-inflammatory therapy for asthma. Annu Rev Med. 1993;44:229-42.
66. Peters SP, Ferguson G, Deniz Y, Reisner C. Uncontrolled asthma: a review of the prevalence, disease burden and options for treatment. Respir Med. 2006 Jul;100(7):1139-51.
67. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nat Rev Immunol. 2011 Aug 5;11(9):607-15. doi: 10.1038/nri3041.
68. Booth FW, Roberts CK, Laye MJ. Lack of exercise is a major cause of chronic diseases. Compr Physiol. 2012 Apr;2(2):1143-211. doi: 10.1002/cphy.c110025.
69. Qin Q, Chen X, Feng J, Qin L, Hu C. Low-intensity aerobic exercise training attenuates airway inflammation and remodeling in a rat model of steroid-resistant asthma. Chin Med J (Engl). 2014;127(17):3058-64.
70. Laidlaw TM, Boyce JA. Cysteinyl leukotriene receptors, old and new; implications for asthma. Clin Exp Allergy. 2012 42:1313–20. doi:10.1111/j. 1365-2222.2012.03982.x
71. Torregrosa Paredes P, Esser J, Admyre C, Nord M, Rahman QK, Lukic A, Rådmark O, Grönneberg R, Grunewald J, Eklund A, Scheynius A, Gabrielsson S. Bronchoalveolar lavage fluid exosomes contribute to cytokine and leukotriene production in allergic asthma. Allergy. 2012 Jul;67(7):911-9. doi: 10.1111/j.1398-9995.2012.02835.x. Epub 2012 May 23.
72. Hallstrand TS, Henderson WR Jr. An update on the role of leukotrienes in asthma. Curr Opin Allergy Clin Immunol. 2010 Feb;10(1):60-6. doi: 10.1097/ACI.0b013e32833489c3.
73. Hammad, H. & Lambrecht, B. N. Recent progress in the biology of airway dendritic cells and implications for understanding the regulation of asthmatic inflammation. J. Allergy Clin. Immunol. 2006 118, 331–336.
74. Pernis AB, Rothman PB. JAK-STAT signaling in asthma. J Clin Invest. 2002 May;109(10):1279-83.
75. Vale K. Targeting the JAK-STAT pathway in the treatment of 'Th2-high' severe asthma. Future Med Chem. 2016;8(4):405-19.
76. Starr R, Willson TA, Viney EM, et al. A family of cytokine-inducible inhibitors of signaling. Nature 1997; 387: 917–921.
77. Elliott J, Johnston JA. SOCS: role in inflammation, allergy and homeostasis. Trends Immunol 2004; 25: 434–440.
78. Sansone P, Bromberg J. Targeting the interleukin-6/Jak/stat pathway in human malignancies. J Clin Oncol 2012; 30(9):1005-14. doi: 10.1200/JCO.2010.31.8907. Epub 2012 Feb 21.
79. Mertens C, Darnell JE., Jr SnapShot: JAK-STAT signaling. Cell. 2007;131:612.
80. Linossi EM, Babon JJ, Hilton DJ, et al. Suppression of cytokine signaling: the SOCS perspective. Cytokine Growth Factor Rev 2013; 24: 241–248.
81. Naka T, Tsutsui H, Fujimoto M, et al. SOCS-1/SSI-1-deficient NKT cells participate in severe hepatitis through dysregulated cross-talk inhibition of IFN-γ and IL-4 signaling in vivo. Immunity 2001; 14: 535–545.
82. Seki Y, Inoue H, Nagata N, et al. SOCS-3 regulates onset and maintenance of TH2-mediated allergic responses. Nat Med 2003; 9: 1047–1054

Instructions for authors of EIR

EIR usually solicits papers from authors with acknowledged expertise in the field to be covered. Unsolicited papers will be considered and can also be accepted. All papers are subject to a peer review process.

Usually the manuscripts will fit into one of two major categories: i. a review which thoroughly covers the area indicated in the heading and includes structuring and critical discussion of existing knowledge and, if possible, the ideas of the authors about potential practical consequences and future developments. Mere mentioning and listing of existing literature is not considered to be a good review. The review can be long, if necessary, or short, if the field covered by the heading is relatively new or very focussed. ii. a paper showing original data accompanied by an extended, review-type discussion.

The general format of the review is somewhat flexible. A review must however have an abstract, an introduction and a conclusion around the main sections. Reviews with three or more sections should list the headings of the sections in form of a bullet point table at the end of the introduction. Longer sections should also give a short interim summary at their end. If substantial amounts of the authors‘ own new data are to be shown, a section on methods and on results must be included. Data will only be accepted, if methods are stated clearly and appropriate statistical evaluation of results is given.

Other types of papers, eg true meta-analyses of a circumscribed sector of literature or papers focussing on new ideas or hypotheses may also be considered. Interested authors, please contact the editorial board.

For reference style use the one as applied by J. Appl. Physiol., with references listed in alphabetical order. In text use ref. numbers in brackets. When giving more than 1 reference in one bracket, use numerical order. The maximum number of references is normally 150.

A short running head should appear after the title, followed by the authors and their respective affiliations. The full address of correspondence should include an e-mail address of the correspondent author. Up to five key words should be added after the abstract.

Please use e-mail for all communications including manuscript submission (word or pdf-file) if possible and paste "EIR” in the subject field of your mailing program. Generally, submission deadline for the next issue of EIR is July, 31th.

Send manuscripts to:
Prof. Dr. Karsten Krüger
Institute of Sport Science, Dept. of Exercise and Health, University of Hannover
Am Moritzwinkel 6,
30167 Hannover, Germany.
karsten.krueger@sportwiss.uni-hannover.de

EDITOR

Karsten Krüger
Leibniz University of Hannover, Germany

ASSOCIATE EDITORS

Jonathan Peake
Queensland University of Technology, Australia

Richard Simpson
The University of Arizona, USA

Neil Walsh
Bangor University, UK

EDITORIAL BOARD

Stéphane Bermon
Monaco Institute of Sports Medicine and Surgery, Monaco

Katsuhiko Suzuki
Waseda University, Japan

David C. Nieman
Appalachian State University, USA

Maree Gleeson
University of Newcastle, Australia

Jeffrey A. Woods
University of Illinois, USA

Frank C. Mooren
University of Giessen, Germany

Lindy Castell
University of Oxford, UK