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Review

# Exercise immunology: Future directions

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**Abstract**

Several decades of research in the area of exercise immunology have shown that the immune system is highly responsive to acute and chronic exercise training. Moderate exercise bouts enhance immunosurveillance and when repeated over time mediate multiple health benefits. Most of the studies prior to 2010 relied on a few targeted outcomes related to immune function. During the past decade, technologic advances have created opportunities for a multi-omics and systems biology approach to exercise immunology. This article provides an overview of metabolomics, lipidomics, and proteomics as they pertain to exercise immunology, with a focus on immunometabolism. This review also summarizes how the composition and diversity of the gut microbiota can be influenced by exercise, with applications to human health and immunity. Exercise-induced improvements in immune function may play a critical role in countering immunosenescence and the development of chronic diseases, and emerging omics technologies will more clearly define the underlying mechanisms. This review summarizes what is currently known regarding a multi-omics approach to exercise immunology and provides future directions for investigators.

**Keywords:** Exercise; Immunology; Lipidomics; Metabolomics; Proteomics

## 1. Introduction

In a recent review[1] of exercise immunology published by the *Journal of Sport and Health Science* (*JSHS*), research discoveries were summarized in several key areas: the acute and chronic effects of exercise on the immune system, clinical benefits of the exercise–immune relationship, nutritional influences on the immune response to exercise, and the exercise effect on immunosenescence. These findings were organized into 4 distinctive time periods, including "2010–future". The observation was made that technologic advances would promote increasing attention in the future to metabolomics, proteomics, lipidomics, gut microbiome characterization, and genomic approaches to exercise immunology.

These multi-omics approaches can be integrated, providing a systemwide view of the multifaceted immune and metabolic response to exercise by simultaneously measuring and identifying large numbers of small-molecule metabolites, lipids, proteins, and other molecules.[2] The ultimate goal of these large datasets is to provide new insights into the interactions between exercise and immune function, with training, nutrition, and health applications applied to the personalized level. At the same time, data integration strategies between these omics disciplines will improve the development of successful precision medicine.[3]

Demanding exercise workloads induce profound perturbations in metabolites, lipid mediators, and proteins that influence immune cell activation, oxygen consumption rates, and function.[1,2] Multi-omics approaches have revealed that metabolism and immunity are functionally coupled, leading to a relatively new area of research called immunometabolism.[4,5] This review provides an overview of immunometabolism and recent findings in the area of systemwide approaches, such as metabolomics, lipidomics, and proteomics because they pertain to exercise immunology. This review also summarizes how the composition and diversity of the gut microbiota can be influenced by a variety of factors, including exercise, and how these factors influence immune function and human health.

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## 2. Immunometabolism: exercise-induced immune cell energy demands

Cellular metabolism and immunology have been linked for more than a half century, with studies examining pathogen-induced changes in immune cell metabolism dating back to at least the mid-1950s.[6] Studies examining the metabolic needs of immune cells continued at a low level in the succeeding decades. However, in the past 10 years there has been an explosion in interest in the field now named *immunometabolism*, and papers reporting new links between cellular metabolic reprogramming and immune cell function can be found routinely in premier scientific journals.

The regulation of immunity by metabolism is complex and has been the subject of many recent comprehensive reviews, but immune cells seem to share several characteristics. Cellular activation in the immune system is almost universally supported by an enhanced program of glycolysis, with glucose metabolism underlying proinflammatory responses in macrophages and dendritic cells[7,8] and Nucleotide-binding oligomerization domain, Leucine rich Repeat and Pyrin domain containing (NLRP3) inflammasome activation,[9] as well as effector functions in cluster of differentiation (CD)4+ and CD8+ T cells and other lymphocytes.[10–13] This upregulation of glycolysis is thought to support a rapid energy demand during immune activation that is not sustainable through fatty acid metabolism[14] and is akin to the increased glucose demand seen in the muscle under maximal exercise.

In contrast, activation of oxidative phosphorylation generally supports anti-inflammatory, memory, and tolerance programs in the immune system. M2 macrophages[15,16] and regulatory T cells[10,17] demonstrate increased oxygen consumption and fatty acid metabolism, as do persistent CD8+ memory T cells.[18] These changes are often characterized by enhanced mitochondrial biogenesis and mitochondrial function[19] and by upregulation of fatty acid oxidation genes such as the fatty acid transporter Carnitine palmitoyltransferase (CPT)1A[20] and are thought to promote longer cellular lifespan and functional programs supporting resolution of inflammation.[14] The pentose phosphate pathway, the tricarboxylic acid (TCA) cycle, and various amino acid metabolic and fatty acid synthetic pathways also play important roles in regulating immune function and determining immune cell phenotype. For an in-depth examination of these pathways, we refer readers to a comprehensive recent review.[14]

From a translational standpoint, aberrant immunometabolism is linked to a variety of disease states. Obesity is characterized by immune infiltration into the adipose tissue, leading to inflammation-induced insulin resistance and, eventually, to diabetes.[21] Infiltrating immune cells in hypertrophic adipose tissue are primarily of the proinflammatory nature, with increases in effector CD4+ and CD8+ and M1-like macrophage subtypes, which rely on glycolytic metabolism and produce large amounts of cytokines, such as tumor necrosis factor-α, interleukin (IL)-1β, and IL-6. Genetic alterations that affect immune cell metabolism also regulate indicators of the metabolic syndrome, such as insulin resistance, as has been shown in macrophages[22,23] and regulatory T cells.[24] Metabolic reprogramming in immune cells additionally contributes to various cardiovascular conditions.[25,26] For example, stimulation of macrophages with oxidized low-density lipoprotein promotes glycolytic activation[27] and induces foam cell formation.[28] Altered immunometabolism has been implicated in many other classes of chronic diseases, notably including cancer[29] and autoimmune disease.[30] Finally, immunometabolic changes in a physiologic aging context have been investigated in several immune cell types, including dendritic cells,[31] monocytes,[32–34] and T cells,[35] and mitochondrial dysfunction in several of these studies has been implicated in or linked to alterations in immune cell functions characteristic of age-related immunosenescence. The link between aging and immunometabolism has been recently reviewed.[36] Given the intimate links between metabolic reprogramming of immune cells and disease progression, interventions that target immunometabolism have tremendous therapeutic potential to treat a variety of diseases.

Despite the fundamental impacts of exercise on metabolism and the well-known effects of exercise on immune function, very little work has been done to date in exercise and immunometabolism. Several studies have shown transcriptional responses consistent with increased mitochondrial function in peripheral blood mononuclear cells from exercised individuals,[37,38] an observation that is consistent with the potential for exercise training to mediate some of its anti-inflammatory effects through polarization of immune cells toward more oxidative phenotypes. This is also supported by a finding that both high-intensity interval training and moderate-intensity continuous training reduce the decrease in mitochondrial function induced by intense exercise.[39] The acute effects of exercise on immunometabolism are largely unknown, although a study has demonstrated that carbohydrate supplementation during exercise may abrogate the characteristic suppression of oxygen consumption after lipopolysaccharide stimulation in monocytes.[40]

Although there is a dearth of literature concerning exercise and immunometabolism, a number of links can be drawn between existing studies and the known effects of physical activity, and they suggest that exercise may be a powerful regulator of metabolism (and, therefore, function) in immune cells. A number of contraction-induced myokines and non-muscle-derived exerkines are known to regulate immunometabolism, although studies demonstrating this have not examined it in an exercise context. Notably, both IL-6 and IL-10 are increased postexercise[41,42] and drive anti-inflammatory responses in macrophages through promotion of pathways leading to increased oxidative metabolism.[43,44] IL-1ra suppresses glucose metabolism in proinflammatory T helper ($T_H$)17 cells to limit their function,[45] and myonectin suppresses macrophage inflammatory responses through inhibition of the cellular metabolic regulator protein kinase B (Akt).[46] Protein exerkines also regulate immunometabolism, including growth differentiation factor-15, which is increased in circulation by exercise[47] and potently enhances oxidative metabolism and alternative activation in macrophages.[48]

Cellular nutrient sensors also play a major role in the regulation of immunometabolism and, thus, exercise has a strong potential to alter immune cell metabolic programs through

alterations in nutrient availability or through direct effects on these intracellular signaling molecules. The 5' adenosine monophosphate-activated protein kinase (AMPK) is activated during low energy availability and is stimulated in muscle and other tissues during exercise.[49] In the immune system, AMPK serves to limit inflammatory activation in macrophages,[50,51] dendritic cells,[8] and T cells.[52] AMPK activity increases fatty acid oxidation in macrophages,[53,54] demonstrating a direct link between immunometabolism, inflammation, and AMPK. AMPK also serves to limit activity of the mechanistic target of rapamycin (mTOR).[55] mTOR is also a cellular energy sensor regulated by exercise[56] and serves in the immune system to activate glycolytic pathways in multiple leukocyte subtypes to enhance inflammatory and effector functions,[57–59] acting in opposition to AMPK. Signaling through the mTOR pathway activates hypoxia-inducible factor-1α (HIF-1α) to enhance glycolysis and inflammation in macrophages[12,60] and T cells.[12,61] HIF-1α is also stimulated by hypoxic conditions during exercise,[62] and hypoxia-induced HIF-1α has been shown to modulate macrophage polarity in adipose tissue.[63] Finally, the Nicotinamide adenine dinucleotide ($NAD^+$) sensor sirtuin 1 (SIRT1) promotes anti-inflammatory and tolerance programs in multiple immune cell types[64–66] and promotes fatty acid oxidation in T cells[67] and myeloid cells.[68] Importantly, the anti-inflammatory effect of AMPK activation in macrophages is mediated at least partly through SIRT1.[50]

Exciting recent evidence has also focused on the role of specific intermediate metabolites in regulating immune function. In particular, byproducts of glycolysis and the Kreb cycle, such as lactate and succinate, respectively, have been shown to alter the activities of multiple immune cell types. Succinate, in addition to its role as a Krebs cycle intermediate, acts as a chemical messenger to induce inflammatory responses,[69] and lactate promotes proinflammatory phenotypes in T cells[70] and promotes an anti-inflammatory phenotype in monocytes.[71] Exercise is well known to promote succinate and lactate accumulation;[72] thus, increases in these metabolites may play a role in regulating immunity. Lactate has also been shown recently to modify histones directly in macrophages,[73] giving it a role in the epigenetic regulation of immune function. Additionally, itaconate, a metabolite produced by the diversion of aconitate out of the Krebs cycle, has recently been described as having potent anti-inflammatory effects in macrophages. To date, no studies have examined the effect of exercise on itaconate; therefore, this molecule offers an immediate opportunity for advancement in immunometabolite and exercise research.

The intersection between exercise immunology and immunometabolism, therefore, offers a number of interesting unanswered questions. Studies addressing the potential link between enhanced mitochondrial function, increased fatty acid oxidation, and anti-inflammatory immune polarization with exercise training are needed, as are explorations of the associations between temporary metabolic reprogramming and transient immunosuppression with exhaustive acute exercise. Exercise may reprogram metabolism in immune cells by secretion of myokines and exerkines, by regulation of cellular energy sensors, by accumulation of immunometabolite regulators, or by other means. Mechanistic knowledge in these areas could lead to clinical trials using exercise (potentially with nutritional or pharmaceutical) strategies to target specific metabolic pathways in immune cells to enhance or suppress their functions, as desired. Additionally, studies addressing immunometabolism in exercise-modifiable diseases, such as obesity, cardiovascular disease, and some cancers, could shed light on mechanisms by which physical activity mediates its known benefits.

## 3. Multi-omics and systemwide approaches

The immune system is responsive to the physiologic stress imposed by the exercise workload. Moderate exercise bouts stimulate the ongoing exchange of immune cells between lymphoid tissues and the circulation, improving immunosurveillance and immune defense activity. The net effect of regular moderate exercise training is a decrease in the prevalence of acute respiratory illness, reduced systemic inflammation, and a delay in the onset of immunosenescence and tumorigenesis.[1,2,74–76] In contrast, high-exercise workloads and the associated physiologic and metabolic stress are linked to transient immune impairment, inflammation, oxidative stress, muscle damage, and an elevated illness risk.[1] Until recently, these exercise-induced effects were measured using a few targeted outcomes, but increasingly, the focus has shifted to multi-omics approaches.[2] This paradigm shift has been driven by exponential advances in measurement technologies and bioinformatics approaches.[3] Data generated from multi-omics approaches will reshape our future understanding of how exercise influences immune function (Fig. 1).

### 3.1. Metabolomics

Metabolomics is the study of small molecular-weight molecules (i.e., metabolites, typically 50–1500 daltons) present in

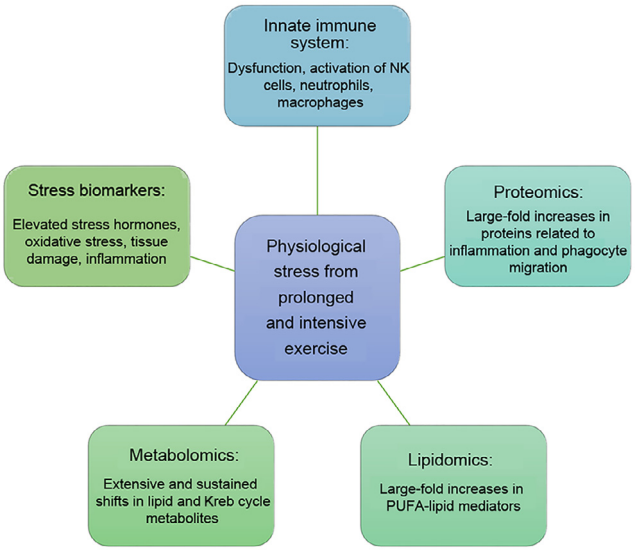


Fig. 1. Immune, stress, and multi-omics approaches to measuring the physiologic stress resulting from prolonged and intensive exercise. NK = natural killer; PUFA = Polyunsaturated fatty acid.

a biologic system.[77,78] The human metabolome comprises more than 114,000 identified and expected metabolites, including peptides and amino acids, lipids, nucleic acids, carbohydrates, organic acids, biogenic amines, vitamins, minerals, xenobiotics and drugs, and other chemicals that humans come into contact with.[79]

Seminal metabolomics studies began in the 1960s, but metabolomics was still considered an emerging field of science as late as 2010 due to technologic advances.[80] High-throughput metabolomics require sensitive mass spectrometry platforms with thousands of chemical standards, rigorous sample extraction and processing procedures, precise quality control and metabolite-identification protocols, and sophisticated bioinformatics support.[77,81] Principal analytic platforms used in metabolomics include nuclear magnetic resonance, gas chromatography spectrometry (GC-MS), and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). LC-MS/MS is the preferred analytic platform and is 10−100 times more sensitive than nuclear magnetic resonance.[82]

A growing number of research groups use metabolomics in exercise-based studies due the widespread availability of mass spectrometry platforms and free access to the Human Metabolome Database (www.HMDB.ca) for interpretation of the data.[40,83−91] Metabolomics-based approaches are particularly valuable in studies combining exercise and nutrition interventions because shifts in hundreds of metabolites from diverse pathways can be measured simultaneously.[2,40,84,86,90] A systematic review of 24 high-quality papers that were published during the past decade revealed that the primary focus of metabolomics-based exercise studies has been on acute metabolite perturbations due to long-duration, high-intensity aerobic exercise.[83] Little information is available regarding metabolite changes coupled with acute moderate bouts of exercise or those associated with long-term exercise training or athletic endeavor.

In a typical study using an LC-MS/MS analytic platform, changes in more than 300 identified metabolites can be measured in plasma samples collected from human athletes exercising intensely for more than 2 h.[40,83,90,91] Large-fold changes have been reported for numerous and diverse lipid-related metabolites during recovery from heavy exertion. This response includes postexercise increases in plasma medium- and long-chain fatty acids, ketone bodies, fatty acid oxidation products, and sulfated bile acids, with decreases in plasma triacylglycerol esters, primary and secondary bile acids, and phospholipids.[83,91] Postexercise increases in lipid-related metabolites are strongly mitigated when overnight-fasted athletes ingest carbohydrate compared to water only.[40,90,91]

Fig. 2 summarizes mean fold increases for 26 metabolites that were selected for a targeted panel to represent the most important metabolites generated by prolonged and intensive exercise.[85,91] These metabolites were identified by bioinformatics procedures using variable importance in projection scores. This targeted panel can be used at a reduced cost for long-duration exercise-based studies when compared to untargeted procedures. All but 1 of the 26 metabolites increasing

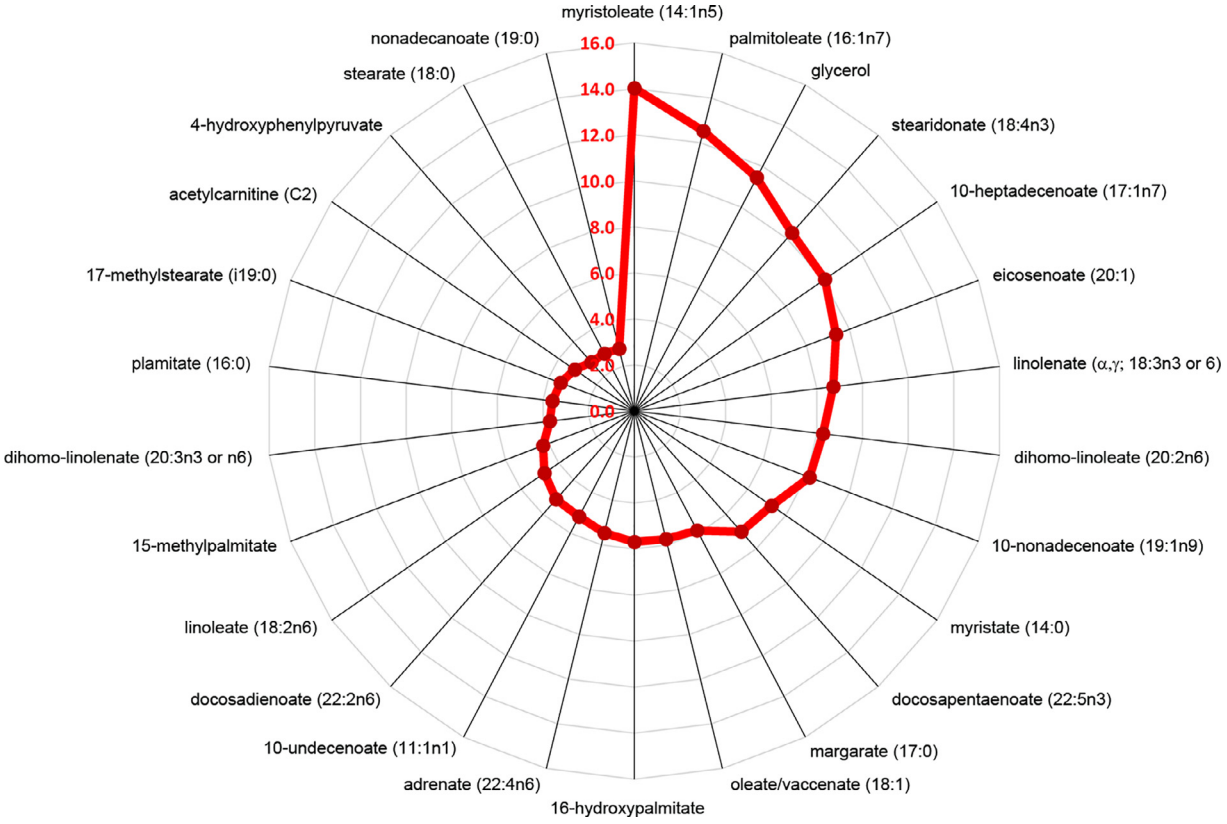


Fig. 2. Mean fold increases for 26 metabolites in a select, targeted panel that represents global metabolite increases induced by prolonged, intensive exercise.[85] This select panel includes metabolites primarily from the lipid super pathway.

postexercise were from the lipid super pathway when considering those with variable importance in projection scores of 2.0 and higher. The parallel increase in plasma glycerol supports an extensive fatty acid mobilization in endurance athletes after intensive endurance exercise bouts. Most of the changes in plasma metabolites after prolonged and intensive exercise reach their nadir within a few hours and have largely abated after 1 day of recovery.

Other exercise-induced plasma metabolite shifts include many types of amino acids and metabolites from the energy TCA cycle, including malate, aconitate, citrate, fumarate, succinate, and alpha-ketoglutarate.[83,91] As emphasized in the immunometabolism section of this article, postexercise increases in plasma TCA metabolites may facilitate immune and inflammation regulation and, thereby, enhance recovery from physiologic stress.[69,73] Plasma amino acid concentrations (e.g., leucine, isoleucine, asparagine, methionine, lysine, and alanine) are generally decreased right after prolonged and intensive exercise, with the exceptions of glutamate, aromatic amino acids, and some urea-cycle-related metabolites such as ornithine. During 1−3 days of recovery from sustained exertion, plasma concentrations of nearly all amino acids increase. These postexercise changes in plasma amino acids support metabolic requirements, including gluconeogenesis and adenosine triphosphate production through the TCA cycle in response to glycogen depletion.[83,85] Postexercise decreases in metabolites related to lysolipid and bile acid metabolism also occur, but the physiologic significance is currently unknown.[83,85] Heavy exertion is associated with significant and sustained decreases in several phosphatidylethanolamines (PEs), a type of phospholipid found primarily in the inner, cytoplasmic lipid bilayer of cell membranes.[85] The acute postexercise decrease in plasma PEs may be viewed as a potential, positive change that over time may chronically decrease tissue PE concentrations.[85]

Immunometabolism has provided new insights into how metabolites influence immune function and many other aspects of normal physiology and pathophysiology.[4,5,25] The large and varied metabolite response to heavy exercise workloads has a direct influence on immune function.[5,22] Exercise-induced shifts in plasma metabolites may reflect changes in the bioenergetic metabolism of immune cells that are central to the immunomodulatory effects experienced during recovery. Metabolites, such as amino acids, biogenic amine hormones, oxylipins, gut-derived phenolics and short-chain fatty acids, lactic acid, glucose, succinate, citrate, aconitate, and steroid hormones, can be used as chemical messengers, fuel, or buffers to control many immune processes, including cell differentiation and activation, immune signaling, inflammation, and pathogen detection and killing.[4,5,92] Direct evidence linking these types of metabolite changes with immune responses to exercise are limited and will be an active area for future research.[40]

### 3.2. *Lipidomics and oxylipins*

Lipidomics is a branch of metabolomics first introduced in 2003.[93] Lipid metabolites are classified into 8 categories, each with its own subclassification hierarchy and identification number (www.lipidmaps.org). The 8 lipid categories include fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides.

Linoleic acid (LA) (18:2n6) is a common polyunsaturated fatty acid (PUFA) in adipose tissue and human diets.[94] After ingestion, LA is converted into longer and more unsaturated fatty acids in the endoplasmic reticulum of cells. Eicosapentaenoic acid (20:5n3) and docosahexaenoic acid (DHA) (22:6n3) are available directly from seafood but can also be synthesized, although to a limited extent, in humans, from α-linolenic acid (ALA) (18:3n3).

Oxylipins are bioactive oxidation products from n6 and n3 PUFAs in the LA and ALA elongation and desaturation pathways.[95−99] Most oxylipins are fatty acyls and are subclassified as eicosanoids, docosanoids, and octadecanoids. The most studied oxylipins are the eicosanoids generated from arachidonic acid (ARA) (20:4n6). Other common oxylipins include the octadecanoids derived from LA and ALA, eicosanoids derived from dihomo-γ-linolenic acid (20:3n6) and eicosapentaenoic acid, and docosanoids derived from adrenic acid (AdA) (22:4n6) and DHA.[96]

Recent advances in mass spectrometry techniques have detected an increasing number of oxylipins, increasing awareness of their vital regulatory roles in many physiologic processes. These roles include regulation of immune function, inflammation, cardiac function and vascular tone, and blood coagulation.[95,98,99] Oxylipins can function as beneficial signaling agents or turn into mediators of immune dysfunction and inflammation, depending on the physiologic context.[95,100] Oxylipins are generated during stress conditions, such as injury or inflammation, in a tightly regulated manner. Membrane phospholipid PUFAs are first released by phospholipase A2 in response to cell activation from stress-related stimuli. The released PUFAs are metabolized by cyclooxygenase, lipoxygenase, and cytochrome P450 (CYP) enzyme systems into numerous and diverse oxylipins.[95,96]

Exercise and diet interventions, obesity, physiologic and environmental stress, and various diseases have marked influences on oxylipin generation.[100−105] The influence of acute and chronic exercise on oxylipin generation is an emerging area of scientific endeavor.[106−112] Exercise-induced muscle tissue injury and inflammation are associated with a rapid and robust innate immune response involving granulocytes, monocytes, and macrophages.[113] Oxylipins are involved in initiating, mediating, and resolving this immune response. Although data are limited, each of the 12 PUFAs in the LA and ALA desaturation pathways is mobilized during intensive and prolonged exercise.[40,90,114,115] A large number of exercise-related oxylipins are produced, many of which are stable enough to be measured in plasma and muscle during several hours of recovery (Fig. 3).[104,110,111]

Future research will help to determine the roles for each type of oxylipin during and after exercise. Both 9- and 13-hydroxy-octadecadienoic acid are stable oxidized metabolites from LA that function as biomarkers for both oxidative stress and inflammation.[115,116] Both 9,10- and 12,13-dihydroxy-

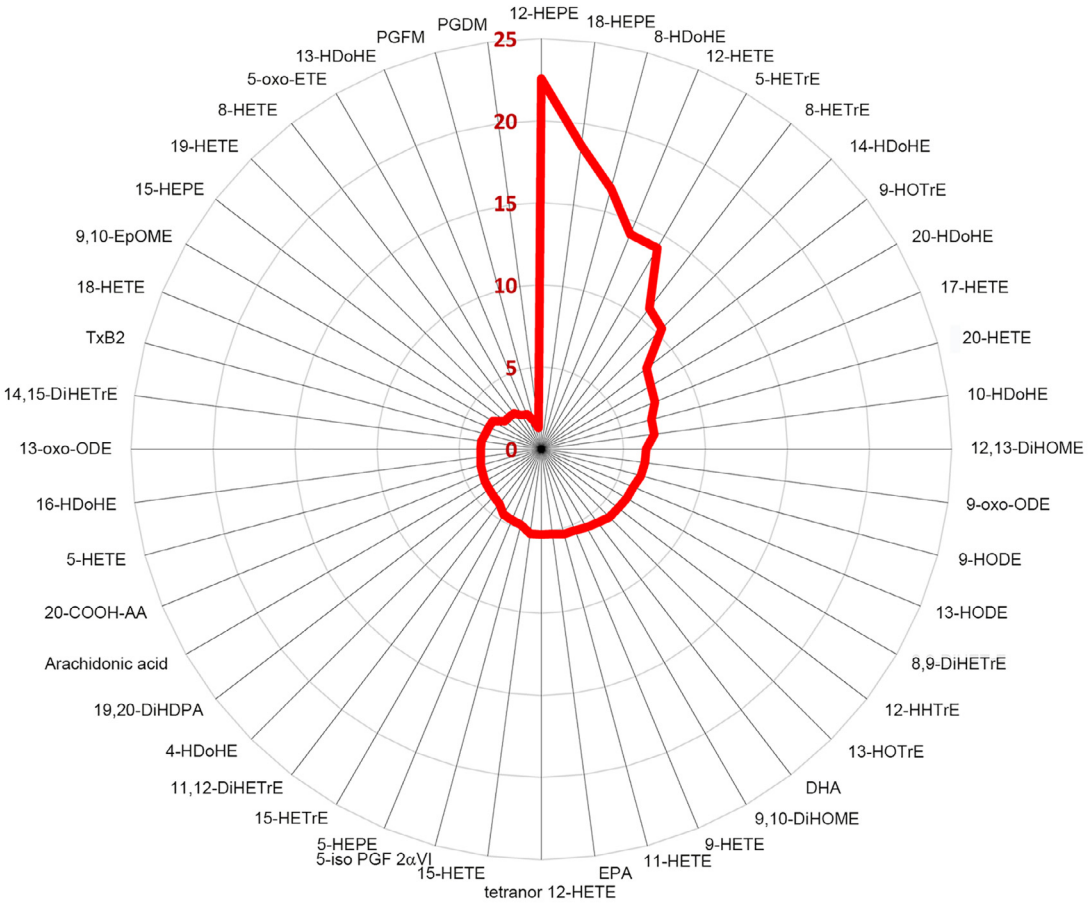


Fig. 3. Exercise-induced increases in oxylipins (ratio values, immediate postexercise/pre-exercise, represented by the red line) for $n = 45$ oxylipins and the substrate fatty acids arachidonic acid (ARA), EPA, and DHA.[104] Blood samples were collected from 20 athletes cycling 75 km in an overnight fasted state. 5-iso PGF2αVI = (8)-5,9α,11α-trihydroxy-prostadienoic acid; 5-oxo-ETE = 5-oxo-eicosatetraenoic acid; 12-HHTrE = 12-hydroxy-heptadecatrienoic acid; 20-COOH-AA = 20-carboxy arachidonic acid; DiHDPA = dihydroxy-docosapentaenoic acid; DiHETrE = dihydroxy-eicosatrienoic acid; DiHOME = dihydroxy-octadecenoic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; EpOME = epoxy-octadecenoic acid; HDoHE = hydroxy-docosahexaenoic acid; HEPE = hydroxy-eicosapentaenoic acid; HETE = hydroxy-eicosatetraenoic acid; HETrE = hydroxy-eicosatrienoic acid; HODE = hydroxy-octadecadienoic acid; HOTrE = hydroxy-octadecatrienoic acid; oxo-ODE = oxooctadecadienoic acid; PGDM = prostaglandin D2 metabolite; PGFM = 13,14-dihydro-15-keto-prostaglandin F2α; TxB2 = thromboxane B2.

octadecenoic acids are peroxisome proliferator-activated receptor ligands with wide-ranging effects, including stimulation of neutrophil chemotactic activity.[115] Prostanoids (e.g., prostaglandin E2 ($PGE_2$), prostaglandin F2 alpha ($PGF_{2\alpha}$)) may exert proinflammatory effects and function as acute signaling molecules for muscle adaptation and regulation of blood flow.[107,117,118] The largest number of oxylipins following heavy exertion come from the n-6 PUFA substrate ARA, and these eicosanoids include at least 15 varieties of hydroxy-eicosatetraenoic acids and dihydroxy-eicosatrienoic acids (DiHETrEs) from lipoxygenase and CYP enzymes.[103,107,110] The hydroxy-eicosatetraenoic acids and DiHETrEs may have multiple potential roles during exercise recovery, including the regulation of leukocyte migration and chemotaxis, macrophage efferocytosis and tissue repair, inflammation, peroxisome proliferator-activated receptor activation, vascular tone, and platelet regulation.[95,119]

Specialized proresolving mediators (SPMs) from n-3 PUFAs include resolvins, protectins, and maresins. SPMs resolve the inflammatory response, stop the recruitment of neutrophils, promote macrophage clearance of debris, and help repair and return the tissue back to homeostasis.[120,121] Lipoxins from ARA are also potent anti-inflammatory and pro-resolving lipid mediators.[120] Studies have not yet consistently shown that SPMs accumulate in the plasma or muscle tissue during and following exercise, but this may be, in part, due to methodologic issues. SPM precursor intermediates have been measured in postexercise plasma and muscle samples.[110,111] For example, exercise increases 18-hydroxy-eicosapentaenoic acid, the resolvin E1 precursor. Although resolvin E1 does not appear to accumulate in human plasma or muscle tissue post exercise, this SPM (if produced) could dampen inflammation and pain.[120,121] Exercise increases plasma and muscle tissue levels of multiple autoxidation products of DHA called hydroxy-docosahexaenoic acids. These may or may not serve as precursors for SPMs, and they function as indicators of oxidative stress.[111,120,121]

Recent studies indicate that CYP-related oxylipins generated from ARA can be influenced by nutritional interventions and represent targets for future studies.[101] Carbohydrate intake

before and during a 75-km cycling time trial strongly countered the mobilization of ARA and the generation of oxylipins, especially those produced through the CYP enzyme system.[110] The carbohydrate effect was especially apparent during the first 3 h of exercise recovery, and the data suggest that phospholipase A2 and CYP enzyme activities were reduced accordingly. The epoxyeicosatrienoic acids are formed by the action of CYP enzymes called epoxygenases on ARA. The epoxyeicosatrienoic acids are rapidly converted to DiHETrEs by soluble epoxide hydrolase. DiHETrEs promote proinflammatory effects and the chemotaxis response of human monocytes to monocyte chemoattractant protein 1 (MCP-1).[122] The countermeasure effect of carbohydrate intake on postexercise plasma levels of DiHETrEs is consistent with other carbohydrate-related anti-inflammatory responses, such as a reduction in IL-6 and blood neutrophil counts.[1,2] Future research will determine whether other nutritional strategies (e.g., flavonoids) mitigate ARA-CYP-generated oxylipins during exercise.

### 3.3. Proteomics

Proteomics is the large-scale study of proteins and the proteome, and early investigations can be traced back to the 1970s.[123] The term *proteomics* was first used in the 1990s. The proteome is defined as the entire set of proteins produced or modified by an organism or system. Proteomics data confirm the presence of a protein that may or may not be translated after expression of distinct genes. Proteogenomics is an emerging field that fuses proteomics with genomics to improve decision making in various disciplines, including precision medicine.[124]

Mass spectrometry procedures are rapidly improving, increasing the number of proteins that can be simultaneously measured in human blood and other body matrixes.[125] Recent advances now allow global proteomics procedures to detect more than 800 proteins using dried blood-spot (DBS) samples from finger-prick blood drops.[126] DBS samples offer many advantages in the athletic and military settings, including ease and safety of transport and handling.[127–129] Proteins in the DBS samples are stable for long time periods at ambient conditions and can be eluted in solvents for later proteomics analysis.

The Universal Protein Resource (www.UniProt.org) is a comprehensive, freely accessible resource for protein annotation and functional information.[130] The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) (www.STRING-db.org) is a biologic database and freely accessible web resource of known and predicted protein–protein interactions.[131] The STRING database contains information on more than 9.6 million proteins from more than 2000 organisms. Protein–protein interaction networks can be visualized by STRING, providing important information on system-level cellular processes and future directions for experimental research.

Exercise-based studies with human subjects using high-throughput proteomics procedures based on mass spectrometry began within the past decade.[127,132–135] Advances in proteomics analysis of muscle tissue, muscle fibers, and the muscle secretome have contributed significantly to uncovering the molecular mechanisms involved in training adaptations.[133] Within the muscle tissue, proteomics-based studies indicate that the primary chronic exercise training response is a greater concentration of proteins from the mitochondrial electron transport chain, tricarboxylic acid cycle, and mitochondrial respiratory chain complex I assembly.[136]

These proteomics-based studies have also shown that skeletal muscle is a major secretory organ during exercise and is 1 means by which health benefits are conferred to the human body.[132,133,136] Proteins are secreted discretely or within extracellular vesicles by skeletal muscle and other tissues into the blood compartment during exercise.[132,135] The nanosized vesicles transfer information to distal tissues and help to regulate physiologic processes. Exercise induces the release of numerous myokines into the circulation, in part through their encapsulation within extracellular vesicles. Whitham et al.[132] showed that a 1-h bout of exercise increased levels of more than 300 proteins in the circulation. Many of them were contained within extracellular vesicles, facilitating cross-talk among tissues.

Blood concentrations of diverse proteins are increased during intensive, prolonged exercise, and many of them are involved in the regulation of immune responses.[127,132,134,137,138] Fig. 4 depicts STRING protein–protein interactions for 29 proteins expressed acutely following a 2.5-h, intensive bout of running or cycling.[121,127] Most of these immune-related proteins are involved with neutrophil function and locomotion and with regulation of the inflammatory response and complement activation. Neutrophils are among the first cells that migrate to inflammatory sites following intensive exercise, and multiple proteins reflect their heightened state of activity, including elastase, S100-A8, S100-S12, defensin, lysozyme, leukocyte elastase inhibitor, and cathelicidin antimicrobial peptide.[127,134,137–139] Postexercise increases in profilin-1 and actin (cytoplasmic 1) support neutrophil actin filament formation that facilitates migration to involved tissues.[127,132]

Some proteins increase chronically following stressful training bouts and can be used as biomarkers of exercise-induced immune activation.[127] These proteins are involved in immune defense and acute phase responses, complement activation and humoral responses mediated by circulating immunoglobulins. The acute phase response is a systemic reaction to stress that includes infection, trauma, and muscle-damaging exercise.[140,141] The acute phase response involves the production of many proteins, including serum amyloid A, complement proteins, C-reactive protein, transport proteins, anti-proteases, and coagulation and fibrinolytic proteins, which can be measured in blood for days following stressful exercise.[127]

## 4. Exercise, gut immune function, and the microbiome

The human gastrointestinal system is colonized by trillions of microbes, and bacterial cells making up the microbiota are thought to be present in the human body at a ratio of approximately 1:1 with host cells.[142] The creation of the Human

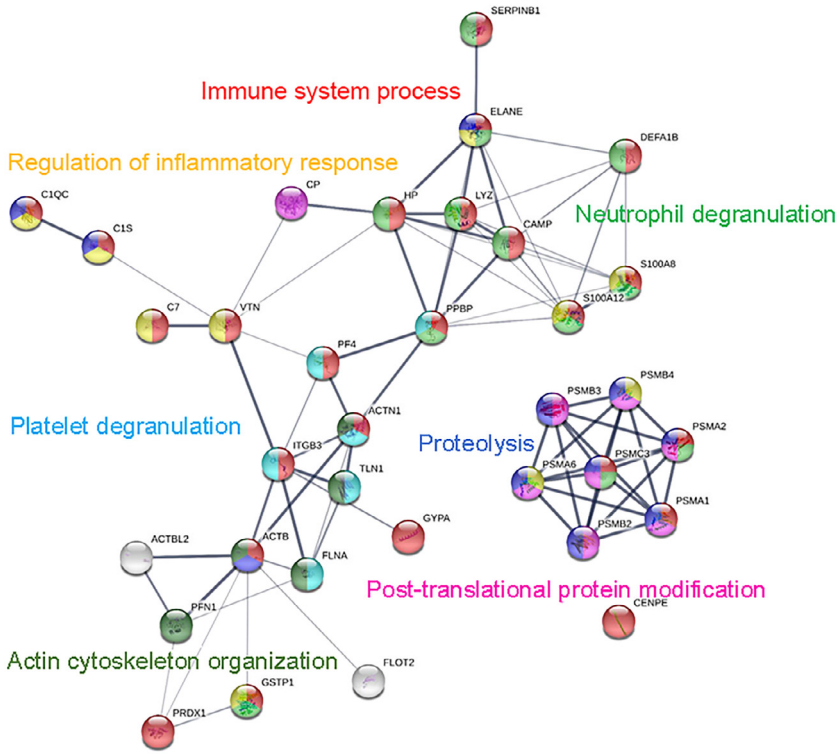


Fig. 4. STRING protein−protein interactions using immune-related proteins ($n = 29$, primary) increasing immediately after running or cycling 2.5 h at 70% $VO_{2max}$.[121] Dried blood-spot samples were collected pre- and postexercise in athletes ($n = 10$) and analyzed using global proteomics procedures. ACTB = actin, cytoplasmic 1; ACTBL2 = beta-actin-like protein 2; ACTN1 = alpha-actinin-1; C1QC = complement C1q subcomponent subunit C; C1S = complement C1s subcomponent; C7 = complement component C7; CAMP = cathelicidin antimicrobial peptide; CENPE = centromere-associated protein E; CP = ceruloplasmin; DEFA1B = defensin, alpha 1B; ELANE = neutrophil elastase; FLNA = filamin-A; FLOT2 = flotillin-2; GSTP1 = glutathione S-transferase P; GYPA = glycophorin-A; HP = haptoglobin; ITGB3 = integrin beta-3; LYZ = lysozyme C; PF4 = platelet factor 4; PFN1 = profilin-1; PPBP = platelet basic protein; PRDX1 = peroxiredoxin-1; PSM = proteasome subunits, types A1, A1, A6, B2, B3, B4, C3; S100A8 = protein S100-A8; S100A12 = protein S100-A12; SERPINB1 = leukocyte elastase inhibitor; STRING, Search Tool for the Retrieval of Interacting Genes/Proteins; TLN1 = talin-1; $VO_{2max}$ = maximal oxygen consumption; VTN = vitronectin.

Microbiome Project (HMP) by the United States National Institutes of Health in 2007 greatly accelerated research in this area, and publications referencing the term *microbiome* now number more than 10,000 per year, compared to the fewer than 400 per year when the HMP began. The dual publications of the large-scale HMP microbiome cohort analysis[143] and the associated framework for microbiome research[144] set off an explosion of sequencing efforts in many laboratories, and alterations to the human microbiome have now been associated with numerous diseases.

Prior to the creation of the HMP, Turnbaugh et al.[145] published a seminal paper demonstrating that the gut microbiota from obese mice and humans undergo changes that include an increase in the ratio of Firmicutes to Bacteroidetes. Transplant of "obese" microbiota also caused lean mice to gain weight and to develop an obese-like phenotype, suggesting that the intestinal microbiota may play a distinct role in disease pathogenesis. Likewise, microbial metabolism of L-carnitine in the gut has been shown to promote atherosclerosis,[146] providing a mechanistic explanation for the link between red-meat consumption and heart disease. The gut microbiome also mediates brain development and anxiety[147] and can potentially affect pathogenesis of Alzheimer's disease,[148] epilepsy,[149] and autism.[150] Microbial diversity is correlated with various immune responses, including in autoimmune diseases,[151,152] allergic responses,[153,154] viral infections,[155] and vaccinations.[156,157] Given these numerous associations between the microbiome and disease, interventions that modulate the gut microbiota to promote health or prevent disease are of obvious interest.

With the publications of the HMP results in 2012 and subsequent prominent papers demonstrating substantial effects on the microbiome of other lifestyle factors such as diet,[158] interest in the effect of exercise on the microbiome accelerated quickly. In 2014, the first notable papers were published detailing exercise effects on gut microbes. Kang et al.[159] demonstrated independent effects of diet and exercise on the microbiome and correlated these shifts to changes in anxiety and cognitive performance in mice. This was preceded by several months by a report that exercise altered bacterial diversity at the phylum level in mice fed high-fat diets,[160] an effect that was speculated to influence the weight loss and decreased adiposity seen with exercise in those mice. The first study to relate exercise with microbial shifts in humans was also published in 2014 by Clarke et al.,[161] who demonstrated in a cross-sectional study that phylum-level differences and increased diversity of gut microbes occur in professional rugby players. In subsequent years, a number of additional cross-sectional studies were published relating exercise to changes in the microbiome in various populations, including people with

type 1 diabetes[162] and in overweight and obese women,[163] postmenopausal women,[164] and others.

The principal limitation of cross-sectional studies of the microbiome is the inability to control easily for other microbiome-modulating behavioral factors such as diet. Several human longitudinal studies have attempted to address this. An examination of microbiome changes during a 3-month longitudinal exercise intervention in breast cancer survivors revealed a relationship between cardiorespiratory fitness and microbial diversity, although exercised and sedentary subjects were pooled for the analysis, and no definitive conclusions were drawn with respect to the efficacy of the exercise intervention in modulating the microbiome.[165] In 2018, several longitudinal exercise studies were published demonstrating the effects of physical activity on the microbiome. In a 6-week training study, Allen et al.[166] demonstrated differential effects on microbial species in previously sedentary adults based on their prestudy status as lean or obese. In this study, fecal butyrate levels increased with exercise only in lean subjects. Likewise, Cronin et al.[167] reported a trend toward changes in microbial diversity in overweight or obese adults after an 8-week exercise intervention, although microbial metabolic pathways appeared largely unaffected. A variety of additional training studies, mostly in rodents, have demonstrated alterations in the microbiome with exercise, and this has been extensively reviewed elsewhere.[168]

Although significant literature now exists demonstrating (sometimes variable) exercise-mediated shifts in gut microbial populations, a number of outstanding questions remain to be answered in this area. The differential effects of various modes of exercise on gut microbial populations are largely unknown, and this may have substantial implications for prescription of exercise interventions targeting the microbiome. Support for the hypothesis that different exercise modalities may have different effects on the microbiome comes from a study by Allen et al.[169] that demonstrated that forced treadmill running (FTR) and voluntary wheel running (VWR) had substantially different effects on the gut microbiota in mice. Similarly, there is now a substantial interest in the effect of exercise on microbial metabolism in the gut, and although several studies have demonstrated exercise-induced increases of the short-chain fatty acid butyrate,[166,170] most exercise studies have focused on microbial sequencing, and the effect of exercise on the levels of other microbial metabolites is still essentially unexplored.

Perhaps the most interesting of currently underexplored questions is whether "exercised" microbiota can be used to treat or cure disease. Fecal microbiota transplantation (FMT) has been used as a treatment for severe *Clostridium difficile* infection for at least 15 years,[171] and modern references to FMT date to the late 1950s,[172] but recently, interest in the use of FMT in the treatment of other diseases has exploded. FMT has shown some efficacy in treating ulcerative colitis and Crohn disease,[173,174] metabolic syndrome,[175] and autism,[176,177] although most conducted studies in these areas used small sample sizes and have not been thoroughly replicated. Nevertheless, given the generally beneficial effects of exercise on the gut microbiome, it is tempting to speculate that gut microbiota from exercised individuals could be transplanted to individuals suffering from various chronic diseases to help alleviate symptoms of those diseases or even to effect a cure.

Some support for this comes from a series of studies of colitis in mice. Cook et al.[178] demonstrated in 2013 that VWR and FTR conferred opposite effects on dextran sodium sulfate-induced colitis, with VWR mediating protective effects, whereas FTR worsened colitis symptoms. Allen et al.[179] followed this up several years later by demonstrating that VWR and FTR have opposing effects on the gut microbiome. Recently, these studies were linked through the demonstration that gnotobiotic mice colonized with microbiota from VWR-exposed mice had reduced symptoms resulting from dextran sodium sulfate-induced colitis compared to gnotobiotic mice transplanted with microbiota from sedentary mice.[180] Likewise, Lai et al.[181] recently demonstrated that obese mice transplanted with microbiota from lean, exercised mice had improved metabolic profiles. These reports suggest that using exercise to "train" the microbiome may be an effective means of boosting the efficacy of therapeutic FMT, although these early results require both replication and translation to human studies.

In recent years, a number of laboratories have demonstrated that various exercise modalities can have modulating effects on gut microbial populations. Although these studies are numerous enough to be convincing, the mechanisms behind these changes, and their implications, are much less clear. In particular, profiling exercise-induced changes in microbial metabolism is likely to yield significant insight into how exercise-induced gut microbiome shifts might impact disease. Likewise, a more thorough investigation into the relative effects of different exercise modalities on microbial populations is necessary for future personalized exercise prescription. Finally, the use of exercise as a pretreatment in donors prior to fecal microbiota transplantation is a promising area of research, but much more study is needed to demonstrate that this strategy would be beneficial, especially in human populations.

## 5. Future opportunities

This review describes how technologic advances and an integrated multi-omics approach using metabolomics, proteomics, lipidomics, and microbiomics approaches have improved our understanding of how exercise influences the immune system and, thereby, impacts health, the aging process, and disease prevalence. A variety of additional emerging technologies for generating large datasets will also be important for future research in exercise immunology. Several laboratories have examined exercise-induced epigenetic changes to peripheral immune cells using genome-wide methylation analysis, and efforts to profile changes in microRNA expression after acute or chronic exercise are also underway.[182] Epigenomic profiling made possible by next-generation sequencing technologies, such as chromatin immunoprecipitation-sequencing (ChIP-Seq,[183] for protein−chromatin interactions), assay for transposase-accessible chromatin using

sequencing,[184] for chromatin accessibility), bisulfite amplicon sequencing for whole-genome methylation,[185] and other continually emerging strategies, will allow exercise immunology researchers to profile intricate subcellular changes to immune cells and to relate them to known functional changes in these cells with exercise. Additionally, newly discovered immune cell phenotypes are now routinely reported by laboratories using technologies such as single-cell RNA sequencing[186] and mass cytometry,[187] and these are soon likely to find applications in exercise immunology studies where exercise-induced alterations in circulating immune cell subsets are of interest.

## 6. Conclusion and applications to health

Each exercise bout promotes the recirculation of key immune cells.[1] Over time, regular exercise training mediates an anti-inflammatory and antioxidant state through multiple mechanisms that remain largely undefined.[74–76] Although many information gaps exist, these exercise-induced, immune-related effects may play a critical role in countering immunosenescence and the development of chronic diseases.[74–76,188–190] Emerging omics technologies for profiling cellular epigenetic, metabolic, and protein expression alterations at high resolution are likely to play key roles in the discovery of the mechanisms behind these changes. In this review we have highlighted some of the key advances in these fields to date, and coordinated efforts such as the National Institutes of Health Common Fund-supported Molecular Transducers of Physical Activity in Humans (MoTrPAC) project[191] are poised to make further important contributions. However, the large number of unanswered questions, some of which we have put forward here, as well as the relatively small number of international scientists working in the area, suggest that there is room for tremendous growth in exercise immunology in the coming years.

## Authors' contributions

DCN and BDP organized and wrote this review. Both authors have approved the final version of the manuscript, and agree with the order of presentation of the authors.

## Competing interests

Both authors declare that they have no competing interests.

## Supplementary materials

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