Exerkines in health, resilience and disease

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Abstract

The health benefits of exercise are well-recognized and are observed across multiple organ systems. These beneficial effects enhance overall resilience, healthspan and longevity. The molecular mechanisms that underlie the beneficial effects of exercise, however, remain poorly understood. Since the discovery in 2000 that muscle contraction releases IL-6, the number of exercise-associated signalling molecules that have been identified has multiplied. Exerkines are defined as signalling moieties released in response to acute and/or chronic exercise, which exert their effects through endocrine, paracrine and/or autocrine pathways. A multitude of organs, cells and tissues release these factors, including skeletal muscle (myokines), the heart (cardiokines), liver (hepatokines), white adipose tissue (adipokines), brown adipose tissue (baptokines) and neurons (neurokines). Exerkines have potential roles in improving

cardiovascular, metabolic, immune and neurological health. As such, exerkines have potential for the treatment of cardiovascular disease, type 2 diabetes mellitus and obesity, and possibly in the facilitation of healthy ageing. This Review summarizes the importance and current state of exerkine research, prevailing challenges and future directions.

[H1] Introduction

Irrefutable evidence supports the importance of physical activity, exercise and cardiorespiratory fitness in the prevention and treatment of chronic diseases, such as cardiovascular disease, obesity, type 2 diabetes mellitus, cognitive decline and many cancers, while enhancing the immune system, healthspan, longevity and resilience **[G]** (**Figure 1**)[1]. Conversely, physical inactivity poses a major public health threat, as it is associated with increased mortality[2] and a notable economic burden[3]. Moreover, the COVID-19 pandemic clearly reinforces the relevance of physical activity for health, due to the effects of COVID-19-related reductions in physical activity[4] and increases in sedentary behaviour, especially due to COVID-19 related quarantine[4]. Moreover, physical inactivity is associated with increased risk of severe COVID-19 outcomes[5].

Although the terms exercise and physical activity are commonly used interchangeably, exercise is typically regarded as intentional physical activity, such as aerobic training[1], resistance training[1] or high-intensity interval training **[G]**[6,7]. By contrast, physical activity encompasses exercise as well as usual occupational and/or domestic activity[1]. Promoting physical activity remains a critical intervention to reduce the incidence and prevalence of common metabolic diseases. In the USA, official guidelines for physical activity were first described in 1995 and recommended that every US adult should accumulate at least 30 minutes of moderate-intensity physical activity on most, preferably all, days of the week[8]. Subsequently, these guidelines have evolved[1]. In 2020, the World Health Organization stated that that all adults should aim for 150–300 minutes of moderate physical activity per week or

75–150 minutes of vigorous physical activity per week or an equivalent combination of moderate-intensity and vigorous-intensity physical activity per week[9]. Despite these recommendations, objective, accelerometer-measured physical activity in the US population reports poor adherence to recommended guidelines, with only 5% of US adults having more than 30 minutes of moderate-intensity physical activity per day[10].

For this Review, we will focus on the potential role of exerkines **[G]** in driving the established benefits of exercise, such as preventing and mitigating disease, promoting health and increasing resilience The term exerkine was coined in 2016[11], although the concept of humoral factors mediating the benefit of exercise has long been recognized. A prime example is lactic acid; its secretion from skeletal muscle was identified over 100 years ago[12]. In 1961, Goldstein et al. speculated about the existence of a non-insulin humoral factor that regulated the effect of exercise on skeletal muscle and liver glucose utilization[13]. For the purposes of this Review, we define an exerkine as a signalling moiety released in response to acute exercise **[G]** and/or chronic exercise **[G]** , exerting their effects through endocrine, paracrine and/or autocrine pathways.

As skeletal muscle comprises approximately one third of body mass and has an important role in exercise[14], the effects of physical activity (**Figure 1**) were initially attributed to blood-borne factors, particularly muscle-secreted hormones (myokines)[15]. Of the myokines, IL-6 has been the most extensively studied since its discovery in 2000[16]. Subsequent exerkine work has broadened to include exercise-related humoral factors arising from the heart (cardiokines), liver (hepatokines), white adipose tissue (WAT, adipokines) and brown adipose tissue (BAT, batokines) and the nervous system (neurokines), with local autocrine (affecting the cell of origin) and paracrine (affecting adjacent cells) effects (**Table 1**). Exerkines are increasingly recognized to include a broad range of signalling moieties, including cytokines, nucleic acids (microRNA **[G]**)[17], mRNA and mitochondrial DNA), lipids and metabolites, which are frequently driven by cell-specific extracellular vesicle secretion[18].

Understanding the role of exerkines in the physiological and biological response to exercise is a principal objective of many investigations sponsored by the National Institutes of Health (NIH)[19], given the demonstrated benefits of exercise enhancing and prolonging human health across the lifespan. In 2020, the National Heart, Lung, and Blood Institute and the National Institute of Diabetes and Digestive and Kidney Disease convened a virtual 2-day public workshop inviting 21 international experts to discuss “Exerkines in Health, Resilience, and Diseases”. The workshop executive summary was published online[20], laying the foundation for this manuscript. In this Review, we summarize the importance and current state of exerkine research, the prevailing challenges and future directions.

[H1] Exercise response variability

[H2] Potential role for exerkines

The majority of exercise research has been limited to studies with genetically homogeneous animal models and/or small numbers of human participants. Moreover, the physiological response to a structured exercise training stimulus remains highly variable in humans and animals owing to a multitude of external and internal factors. In terms of external factors, the context of exercise matters, as exercise timing relative to circadian rhythms[21], fed–fasting status[22] or post-exercise dietary composition[23] might influence metabolic outcomes. This variability is well-detailed in a study published in 2022, which included an atlas describing the time-dependent effects of exercise across multiple tissues after a single-bout of treadmill exercise[24]. Addressing these external factors will require careful consideration of the exercise exposure, controlling the exercise exposure’s environmental context and serial sampling of blood and tissue prior, during and after exercise. This level of rigor is needed to ‘reduce the noise’ and facilitate interpretation of the temporal signatures of circulating and tissue-specific exerkines. In terms of internal factors, genetics have a critical role in the response to exercise. The HEalth, RIsk factors, exercise Training And GEnetics (HERITAGE) Family Study involved

20 weeks of supervised aerobic exercise training of 481 sedentary, healthy, white adults from 98 two-generation families and reported that the maximal heritability estimate for the aerobic capacity response was roughly 47% (Ref. [25]). Furthermore, chronic exercise training elicited a 'non-response' in terms of improved aerobic capacity in ~20% of individuals[25]. In addition, 7–15% of individuals demonstrated an 'adverse response' regarding alterations in systolic blood pressure, as well as in fasting levels of HDL-C, triglycerides and insulin[25,26].

To drive the application of precision medicine to exercise, investigations into the mechanisms underlying the response variability to exercise are sorely needed. The contribution of exerkines to the variation in exercise response remains under active research and will be a key focus of the Molecular Transducers of Physical Activity Consortium (MoTrPAC: NCT03960827). This NIH-supported research consortium is designed to discover and broadly characterize the range of molecular transducers that underlie the variable effects of exercise in humans and animals. For humans, MoTrPAC has several unique features: its size (~2,600 participants); its recruitment of sedentary participants who will undergo a 12 week programme of either aerobic exercise, resistance exercise or control, with comparison to a reference group of highly active endurance exercise or resistance exercise participants; and its time-course analysis of changes in tissue (muscle and adipose tissue) and plasma metabolites[19]. In animals (*n* of ~800 studied), the unique feature of MoTrPAC will be its focus on detailed biospecimen analysis across multiple time points and organs, which cannot be easily replicated in humans, in young (6 months) and old (18 months) male and female rats after an acute (single session) bout of treadmill exercise, or after chronic treadmill exercise (8 week duration) versus non-exercised control mice[19]. Identification of an exerkine or a panel of exerkines, which capture the benefits of exercise, would have potential implications for improving the health of those unable to exercise, such as those with ageing-associated exercise intolerance.

[H2] Influence of exercise exposure

Exerkines are secreted in response to acute exercise, which is usually a single episode of either aerobic or resistance exercise. Chronic exercise is also associated with altered humoral factors, even in the resting state, suggesting that exerkine alterations can reflect chronic training effects[27].

The acute exerkine response is influenced by the type of exercise, duration of exercise, underlying fitness, fed–fasting status and sample timing after exercise. In a human model, the blood concentration of glucose typically remains stable during acute exercise, with the liver releasing glucose for brain and skeletal muscle usage[28]. During exercise, skeletal muscle also uses lipid as fuel, which originates from the triglycerides stored in muscle and free fatty acids (FFAs) released from WAT[29]. The classic exerkines released during acute exercise, as reported in human and animal models, include IL-6, IL-8, IL-1 receptor antagonist (IL-1RA) and IL-10. In a human study where blood samples were collected before and after a marathon, plasma levels of several cytokines (IL-6, IL-1RA, IL-10 and tumour necrosis factor (TNF)) were higher than baseline levels when collected immediately post-exercise, peaking 1–2 hours post-exercise and remaining elevated for several hours (~4 hours) post-exercise[30]. Certainly, the type and intensity of exercise matters. As an example, the exerkine response to high-intensity interval training depends on exercise intensity; higher exercise intensity corresponds with higher plasma levels of IL-6, while IL-10 levels remain unchanged compared with pre-exercise[6]. **Supplementary Table 1** reports examples of singular exerkine alterations. Currently, exerkine research is evolving from measuring singular exerkine changes to characterizing metabolic profiles[31], of which challenges in analysis and interpretation remain (**Table 2**).

Notably, the acute exerkine response does not necessarily parallel the chronic exerkine response (**Supplementary Table 1**). Typically, acute exercise exposure is associated with responses focused on the maintenance of metabolic homeostasis, with acute inflammation balanced by anti-inflammatory mediators[30] and accommodating shifts in fuel utilization. By contrast, chronic exercise exposure is associated with responses focused on long-term

metabolic adaptations and decreased inflammation[27]. However, when investigating chronic exercise exposure, the caveats of recent acute exercise, recent dietary composition, underlying fitness, fed–fasting status, circadian timing and training modality needs to be considered. Moreover, the effect of exercise could also be influenced by alterations at the level of the exerkine receptor, in addition to alterations in plasma levels of exerkines. For example, in humans, chronic exercise training reduces plasma concentrations of IL-6; however, this effect could be partially mitigated by increased skeletal muscle mRNA expression of IL-6 receptor[32].

[H1] Exerkines: technical considerations

[H2] Discovery techniques

Exerkine research is increasingly focused on measuring changes across a broad swath of factors rather than singular change. Specifically, interest is increasing in '-omics' technology to capture exercise-related changes in lipids (lipidomics), metabolites (metabolomics), proteins (proteomics), gene expression (transcriptomics) and DNA alterations (epigenomics) (**Table 2**)[31]. A paper in 2020 studied humans across the spectrum of insulin sensitivity (*n* = 36, ranging from people with high insulin sensitivity to patients with diabetes mellitus) who performed an acute bout of treadmill-based exercise to reach peak oxygen uptake. This exercise exposure altered >50% of measured molecules, spanning platforms based on lipidomics, metabolomics, proteomics, transcriptomics and epigenomics[31]. **Table 2** describes commonly used platforms for exerkine analysis, including their relative advantages and disadvantages. Mass spectrometry is often used for targeted and untargeted -omics analysis, whereas immunoassays are commonly used for analysis of proteins and metabolites. Genetic analyses include RNA-sequencing, methylation-sequencing (Methyl Seq) and Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-Seq)[33]. Together, these platforms provide a rich profile of the molecular and epigenomic changes that occur in response to acute and chronic exercise.

[H2] Extracellular vesicles

In the exerkine field, interest is also intensifying in the role of extracellular vesicles as important carriers of molecular signals and drivers of inter-organ crosstalk related to exercise[18]. Extracellular vesicles are membranous structures that are released from almost all cell types, with cell-specific profiles. They vary in size, ranging from 150 nm to 1,000 nm, and carry an assortment of material, including proteins, nucleic acids and lipids[18,34]. The content of extracellular vesicles reflects the unique and varied composition of the cells from which they are released. As an example of extracellular vesicles acting as an exerkine in humans, acute exercise increases plasma levels of various microRNAs post-exercise and chronic exercise increases various microRNAs in the resting state[17], supporting the possibility of microRNAs exerting their endocrine effects via extracellular vesicles-based transport[11,34].

Extracellular vesicles can be routinely isolated and profiled from cell culture media. Studying the molecular cargo of plasma-derived extracellular vesicles, however, remains uniquely challenging. Critical to extracellular vesicle analysis is the careful consideration of pre-analytical steps, including proper collection and isolation. Isolation techniques include ultracentrifugation (using a differential density gradient), ultrafiltration, size exclusion chromatography, high-resolution mass spectrometry, capillary electrophoresis, asymmetric-flow field-flow fractionation and immunoaffinity capture[35]. Moreover, contamination at the time of collection needs to be considered as extrecellular vesicles can arise from *ex vivo* platelet activation[36,37]. A 2021 paper presented an optimized size-exclusion-chromatography method for proteomic analysis of plasma-derived extracellular vesicles from platelet-poor plasma; this technique had greater precision relative to conventional extracellular vesicle techniques and demonstrated a distinct exosome protein cargo profile post-acute exercise in humans[37].

[H2] Autocrine, paracrine and/or endocrine effects

Initially, exerkine research focused on changes in plasma levels of cytokines, especially before and after an acute exercise exposure[30]. The classic exerkines are cytokines, of which IL-6 has been the most extensively studied since its identification as a myokine in 2000 (Ref. [16]). Subsequently, the field evolved into examining the endocrine effects of exerkines, where molecules secreted from the source tissue, traditionally viewed as skeletal muscle, affect distant tissues[15]. Especially within the past 15 years, interest is increasing in the local effect of exerkines, either on the secreting tissue (autocrine) or the adjacent environment (paracrine)[38] (**Table 1**), non-muscle exerkine sources[39], **(Supplemental Table 1**) and exerkine profiling rather than singular exerkine alterations (**Table 2**)[31,40].

A common perception among the general scientific community is the view of exerkines as a cytokine exerting its effects in an endocrine fashion, affecting tissues distant from the originating tissue. Exerkines are not merely cytokines, however, as hormones, neurotransmitters or metabolites associated with exercise, such as catecholamines[41], lactate[42] or free fatty acids[29] could also serve as exerkines with endocrine signalling potential[43].

From an autocrine standpoint, exerkines affect their origin cells by coupling local energy balance to tissue growth and metabolic homeostasis (**Table 1**). For example, in skeletal muscle, myocytes secrete factors such as lactate[42], musclin[44] and myostatin[45] that couple exercise to changes in mitochondrial biogenesis and myocyte substrate utilization.

Muscle and other highly metabolically active tissues can also secrete exerkines that exert local (paracrine) effects[38]. For example, muscle secretes vascular endothelial growth factor (VEGF)[46,47], angiopoietin 1 (Ref. [48]) and IL-8 (Ref. [49]) to regulate tissue angiogenesis, modulate blood flow and increase nutrient availability to support tissue growth (**Table 1**)[27,50,51,52,53]. Exercise-related paracrine effects are also observed in the nervous system[54], adipose tissue[43,55-59], bone[60-62], cartilage[63], extracellular matrix[64,65] and the immune system[15,55-57,60-62,63-65,66,67].

[H1] Tissue-specific exerkine relationships

[H2] The cardiovascular system

Physical activity reduces the risk of cardiometabolic disease and mortality. Although exercise mitigates traditional cardiovascular risk factors, such as obesity or dyslipidaemia, these benefits incompletely account for the effects of exercise on cardiometabolic health. Both human and animal studies support a role for exerkines potentially enhancing cardiometabolic health (**Figure 2**, **Table 3**, **Supplementary Table 1**). Exerkines could also oppose multiple mechanisms associated with cardiovascular disease, such as persistent systemic inflammation, dysregulated energy balance and fuel utilization. Furthermore, the enhanced angiogenesis associated with certain exerkines could mitigate ischaemia. Notably, exercise might improve endothelial function. As the vascular endothelium lies at the interface between blood and tissue, its wide-ranging distribution and strategic positioning supports its potential role as an initiator and recipient of exerkine-related effects. For example, interplay between the endothelium and established exerkines, such as nitric oxide[53] and VEGF[27], has been shown to influence vascular tone, inflammation, regeneration and thrombosis, and has an important role in cardiovascular and overall resilience[68].

Contracting skeletal muscle produces many molecules that can enhance the cardiovascular system. Studies in humans[15,46,69-75] and animal models[15,44,50,76,77] have shown that angiopoietin 1 (Ref. [50,69,70]), fractalkine[71,72], fibroblast growth factor 21 (FGF21)[73,74], IL-6 (Ref. [15]), IL-8 (Ref. [51,75]), musclin[44], myonectin[76] and VEGF[27,46,77]. These exerkines are generally increased with acute exercise; however, the exerkine response to chronic training, as measured by assessing exerkines in plasma during the resting state, can be quite variable and discrepant from the acute effects. As shown in **Table 3** and **Supplementary Table 1**, examples include angiopoietin[69,70], FGF21 (Ref. [74,78]), fractalkine[71], IL-6 (Ref. [30,79]) and IL-8 (Ref. [51,75]).

[H2] Adipose tissue

Exercise facilitates WAT lipolysis to provide FFA for fuel utilization[29]. Although this lipolysis was typically attributed to adrenaline release[41], acute exercise in humans also releases additional molecules[80], such as growth differentiation factor 15 (GDF15)[81] and IL-6 (Ref. [82]), which also affect lipolysis (**Table 3**; **Supplementary Table 1**). Lipolysis is not the only avenue by which exerkines can affect adipose tissue mass. For example, in myonectin knockout mice, WAT lipolysis was unaffected, however, dietary lipid clearance was impaired compared with wild-type mice, resulting in increased WAT mass and decreased liver steatosis[83].

A potential effect of exercise on WAT is 'browning', where WAT increases mitochondrial content, metabolic rate and heat production. WAT browning might have metabolic importance, as individuals with PET–CT-defined BAT had a decreased prevalence of cardiometabolic disease, particularly if they had overweight or obesity[84]. In a mouse model, fibronectin type III domain containing 5 (FNDC5) is cleaved in the muscle cell and secreted as irisin, which induces WAT browning to increase energy expenditure and consequently reduce obesity[85]. Transgenic mice that overexpress muscle peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) have higher circulating levels of irisin and increased WAT browning compared with control mice[85]. Hence, the initial excitement regarding irisin as an exerkine, as exercise generally increases muscle *PGC1A* expression in both animal[86] and human models[87].

As the irisin findings and exercise-induced browning of WAT concepts were reevaluated in humans, the initial excitement has been subsequently tempered. Although acute exercise in humans generally increases plasma levels of irisin[88], the effect of chronic exercise training remains highly variable. A meta-analysis of several randomized controlled trials even reported lower levels of irisin post-training than pre-training[89]. Trained athletes have lower BAT activity and no difference in WAT browning markers compared with lean, sedentary men[90]; this observation is supported by a human study of chronic exercise training, which did not observe any browning of WAT (as assessed by biopsy)[91]. Thus, whether exercise can brown WAT in humans, especially through an irisin-mediated pathway, remains controversial[56,84,85,91].

Adipose tissue can also secrete exerkines. A prime example is 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME), which is secreted from BAT and increases skeletal muscle oxidative capacity[93]. In humans, circulating levels of 12,13-diHOME are inversely associated with adipose tissue mass, fasting blood levels of insulin and blood levels of triacylglycerol[94]. A 2021 study showed that BAT transplantation in mice increased plasma levels of 12,13-diHOME and improved cardiac haemodynamics[95]. These data suggested that a sustained increase in plasma levels of 12,13-diHOME preserved cardiac function and remodelling and increased cardiac haemodynamics through a direct effect on the cardiomyocyte. These findings were reinforced by observations in humans, where the presence of cardiovascular disease was associated with decreased plasma levels of 12,13-diHOME[95].

Interestingly, skeletal muscle can influence the adipose tissue response to exercise via lactate secretion. The prototypical example is transforming growth factor β2 (TGFβ2)[43]. In an animal model, specific lactate exposure *in vitro a*nd *in viv*o increased adipocyte expression of TGFβ2 (ref.[43]). Furthermore, the same study found that in an animal model of chronic exercise, increased adipocyte levels of TGFβ2 expression and secretion were associated with improvements of glucose metabolism, lipid oxidation and a possible reduction of adipose tissue inflammation. Parallel findings were also observed in humans undertaking chronic exercise, albeit less pronounced than the animal model observations[43]. Nevertheless, these findings demonstrate the possibility of lactate mediating tissue-to-tissue communication during exercise.

[H2] Skeletal muscle

Exerkines originating from multiple tissues have demonstrated the capacity to improve skeletal muscle function and growth (**Table 3**; **Supplementary Table 1**). Apelin is an example of a myokine affecting muscle function. In both humans and animal models, exercise increases levels of apelin[96-98]. In an animal model, skeletal muscle[97,98] serves as a source of apelin secretion, which improved skeletal muscle function in the setting of ageing, supporting the

potential of apelin as a therapeutic to combat age-related sarcopenia[96-98]. Specifically in old mice, increased apelin exposure (by daily injection or skeletal muscle overexpression) stimulated muscle mitochondrial biogenesis, muscle protein synthesis and enhancement of muscle stem cells to stimulate muscle regeneration[98].

12,13-diHOME is an example of a batokine with muscle effects. In both humans and animal models, exercise facilitates BAT secretion of 12,13-diHOME, which enhances skeletal muscle FFA uptake and oxidation[93]. The hepatokines follistatin and fetuin-A also affect muscle function. For example, in both humans[99-101] and animal models, acute exercise[99,102] and chronic exercise[100] increase liver-secreted follistatin to decrease the serum level of myostatin. Decreased levels of myostatin enhance skeletal muscle growth and improve whole body glycaemic control[45,101]. Furthermore, fetuin-A worsens peripheral insulin resistance by reducing insulin signalling and glucose transporter type 4 trafficking[39]. Although acute exercise in humans does not alter plasma levels of fetuin-A [99], chronic exercise might decrease plasma levels of fetuin-A [73,103]. Notably, in humans, the lowering of levels of fetuin-A by exercise training correlated with decreased hepatic insulin resistance, as measured by the hyperinsulinaemic–euglycaemic clamp[103]. Additional exerkines involved in muscle growth and development include the following: IL-7 (Ref. [104]), IL-15 (Ref. [101]), follistatin[99], leukemia inhibitory factor[105], syndecan-4[106] and myostatin[45,74].

[H2] The liver and the gut

Exercise reduces hepatic steatosis independently of weight loss[107]. The liver is recognized as a source for many circulating proteins, with ~2,500 liver-secreted proteins identified using modern liquid chromatography and mass spectroscopy technologies[39]. Not surprisingly, the liver is the source of many acute exercise-responsive cytokines (**Supplementary Table 1**). These exerkines affect glucose and/or lipid metabolism (for example, angiopoietin-like protein 4 in humans and animal models[39,80]), browning of WAT

(FGF21 in an animal model[92]), lipolysis (FGF21 in humans and an animal model[78]) and the maintenance of cellular homeostasis (Heat Shock Protein-72 in humans[108]).

Exercise also alters the gut microbiome[109]. Chronic exercise in humans and animal models alters the composition and functional capacity of the gut microbiota, independently of diet; these exercise-dependent microbiota changes might be independent of weight while being contingent on exercise intensity, modality and sustainment[109]. In humans, chronic exercise altered the gut microbiome to increase availability of short chain fatty acids, particularly butyrate[110]. Once these participants ceased training, exercise-induced changes in the microbiota were largely reversed when re-measured after a 6-week sedentary period[110]. The mechanisms by which exercise might alter the gut microbiome remain numerous, including altering the gene expression of intraepithelial lymphocytes for a more favourable inflammatory profile[111], influencing blood flow in the gut[112] or changing bile acid excretion[113].

[H2] The endocrine system

As exercise has established benefits in improving dysglycaemia, this section focuses specifically on exerkines affecting glucose homeostasis (**Table 3**; **Supplementary Table 1**). In humans, circulating levels of B-aminoisobutyric acid (BAIBA) increased with chronic training[56] and inversely correlated with insulin resistance[56]. In wild-type mice, BAIBA treatment reduced insulin resistance and suppressed inflammation[56]. Another study using C2C12 mouse myocytes and a wild-type mouse model (palmitate or high-fat diet exposure) found that BAIBA treatment attenuated insulin resistance, reduced inflammation and increased fatty acid oxidation through AMP-activated protein kinase (AMPK) and a AMPK–PPARδ-dependent pathway in skeletal muscle[114]. Limited human data showed that an acute exercise bout increases plasma and muscle expression levels of fractalkine (encoded by *CX3CL1*)[71], which is a chemokine that favourably regulates glucose-stimulated insulin secretion by enhancing β-cell function[115].

Chronic exercise in humans also reduces circulating levels of fetulin-A[73,103]. Fetulin-A has been shown to impair β-cell sensing by reducing glucose-stimulated insulin secretion[116].

In humans, both acute exercise[99] and chronic exercise[100] increased circulating levels of follistatin. The extent to which follistatin might improve glycaemic measures remains controversial. After bariatric surgery, improvements in HbA_{1c} have been observed in the setting of reduced levels of follistatin[117]. Furthermore, inactivating hepatic follistatin in a mouse model improved WAT sensitivity and reduced hepatic glucose production[117]. *In vitro*, irisin prevented excessive lipogenesis of mouse islets under glucolipotoxic conditions, resulting in improved insulin secretion, inhibition of apoptosis and restored β-cell function-related gene expression[118]. The myokine IL-6 is also associated with favourable alterations in glucose homeostasis. In humans, IL-6 infusion delayed gastric emptying and lowered postprandial glucose levels[119]. In rodents, increasing IL-6 by exercise or by IL-6 injection increased the production of glucagon-like peptide 1 by intestinal L cells and pancreatic α-cells to enhance glucose-stimulated insulin secretion. These benefits of IL-6 on enhancing insulin secretion were seen across multiple rodent models of T2DM[120]. In healthy humans, IL-6 infusion to levels similar to those seen with strenuous exercise enhanced insulin-stimulated glucose-uptake but did not alter whole body lipolysis or lipid oxidation[121]. However, another study of IL-6 infusion into humans reported that IL-6 stimulated lipolysis and lipid oxidation[55,122]. These seemingly conflicting findings warrant further research to establish the effect of exerkines on glucose metabolism.

[H2] The immune system

The broad effects of exercise on immune function implicate mobilization and altered function of cytokines and immune cells, such as neutrophils, leukocytes and natural killer cells. (**Figure 3**, **Supplementary Table 1**)[30,123,124]. The effects of chronic exercise on the immune system might depend on intensity, with immune enhancement by moderate exercise and possible impairment by strenuous exercise[125]. As shown in a human model, an acute bout of

exercise might be initially proinflammatory, but subsequently this effect is offset by an anti-inflammatory response[30,125]. The exercise-induced acute increase in circulating levels of IL-6 increases plasma levels of anti-inflammatory cytokines, such as IL-1RA and IL-10 (Ref.[126]). IL-1RA inhibits IL-1β signal transduction[127], whereas IL-10 inhibits production of proinflammatory cytokines, such as TNF[128]. In healthy humans, one bout of exercise or an IL-6 infusion blunted the increase in circulating levels of TNF induced by infusion of lipopolysaccharide[129]. Thus, an acute bout of exercise induces anti-inflammatory effects that might in part be mediated by IL-6, possibly in conjunction with other known anti-inflammatory factors, such as adrenaline and cortisol[123]. As a pleiotropic factor, the effect of IL-6 on metabolism and inflammation remains context-dependent. Although IL-6 is transiently increased after acute exercise, the baseline (or 'resting') circulating levels of IL-6 are lower in exercise-trained individuals than in untrained individuals[79]. Future studies focusing on tissue and cell type-specific effects as well as different exercise regimens will help delineate the temporal and spatial requirement of IL-6 in mediating exercise benefits.

An emerging frontier in exercise biology involves exerkine-induced immune effects in increasing resilience to cancer or as co-adjuvant to cancer therapy. The anti-cancer effects of exercise might not be limited to its effect on body weight. A meta-analysis pooled data from 12 prospective cohorts with self-reported physical activity and found that increased physical activity levels are associated with decreased risk of incident cancer across multiple types; many of these associations remained even after adjusting for BMI[130]. Acute exercise creates a unique 'exerkine' milieu, lasting several hours after exercise cessation, which provides a temporal window for immune function stimulation[31]. For this reason, exercise could potentially serve as a co-adjuvant treatment for cancer therapy. In tumour-bearing mouse models (across five tumour models), mice that undertook voluntary wheel running had a greater than 60% reduction in tumour incidence and tumour growth compared with sedentary mice. Further analysis of these mouse models showed that adrenaline and IL-6 induced natural killer cell mobilization,

redistribution and tumour infiltration to inhibit tumour growth[124]. Another mouse model found that exercise metabolites such as lactate and possibly tricarboxylic acid intermediates enhance the antitumour effector profile of $CD8^+$ lymphocytes[131]. Of note, exercise is associated with enhanced secreted protein acidic and rich in cysteine (SPARC) secretion in humans and animal models[132]; this matricellular protein regulates cell function and tissue remodelling, while inhibiting proliferation and promoting apoptosis of colon cancer cells[133].

Crosstalk exists between skeletal muscle and the immune system. Contemporary views toward skeletal muscle now consider muscle as an immunoregulatory organ, especially affecting lymphocyte and neutrophil trafficking and inflammation. During acute exercise, immune cells are mobilized by muscle exerkine secretion, such as fractalkine to enhance regeneration (in humans)[134] or meteorin-like (METRNL) to increase beige adipose tissue thermogenesis (animal model)[135]. As previously noted, one bout of exercise or an IL-6 infusion in humans blunts the increase in circulating levels of TNF that are induced by lipopolysaccharide infusion[129].

IL-13 is an important main T helper 2 cell (T_H2) cytokine that mediates the anti-inflammatory polarization of resident macrophages in WAT[136]. IL-13 is an also an exerkine, increasing in the circulation after exercise training in humans and mice[137]. Mice lacking IL-13 show reduced running capacity and lose certain beneficial effects of exercise training, such as improvements in glucose tolerance and endurance running capacity[137]. Unlike IL-6, IL-13 is produced by type 2 innate lymphoid cells (ILC2s) in skeletal muscle. As IL-13 deficient mice showed defective muscle fatty acid utilization after acute exercise and failed to increase muscle mitochondrial biogenesis after chronic exercise, the ILC2 to IL-13 axis might have an important role in the metabolic adaptation to exercise training[137]. Interestingly, ILC2 and T_H2 cytokines also control beige adipocyte recruitment in rodents[138], suggesting that ILC2 to T_H2 signalling might partially mediate exercise-induced beiging of WAT. Identifying which stimulants within skeletal muscle or WAT activate ILC2s during exercise remains under investigation.

[H2] The nervous system

Exercise is a promising non-pharmacological strategy to maintain and improve brain function[139]. **Figure 4** presents an overview of the purported effects of exercise on the nervous system (**Supplementary Table 1**). Of note, evidence for the benefits of exercise on cognition remains variable, probably owing to the lack of randomized controlled trials throughout the lifespan with standardized exercise interventions and comparable methods for cognitive assessment[140,141,142,143]. The effects of exercise on the brain are most apparent in the hippocampus, a part of the brain involved in learning and memory[144]. In older adults (aged 55–80 years), participation in an aerobic walking programme increased hippocampal volume and improved memory[145]. Moreover, accumulating evidence suggests that physical activity, as noted by preclinical, observational and interventional studies in humans, could prevent or delay the onset of neurodegenerative conditions[139]. In humans, acute exercise increases plasma levels of brain-derived neurotrophic factor (BDNF)[7], whereas chronic exercise training has been shown to either not alter[141], to increase or to decrease plasma levels of BDNF[141,146]. In rodents, chronic exercise upregulates BDNF in the hippocampus, which is essential for adult hippocampal neurogenesis and neural plasticity[147]. Chronic exercise in rodents also enhances hippocampal synaptic plasticity, adult neurogenesis and neurotrophin levels, as well as memory function[148]. In addition, voluntary wheel running in rodents increases the number of new hippocampal neurons, enhances morphological maturation, such as dendritic branching and spine density, and alters the circuitry of adult-born neurons[148].

There is increasing recognition that peripheral factors that might trigger the effects of exercise on the brain. In the past 20 years, researchers begun to test the hypothesis that metabolites, peptides and proteins released from liver, adipocytes, blood cells (particularly platelets) and muscle might influence the central nervous system[139,149,150]. Since 2020, several studies have transferred plasma from exercised animals into sedentary animals, with

subsequent improvements in cognitive function, supporting the presence of a transferrable factor in improving cognitive function[149,150]. Evidence is now accumulating that factors released from non-neuronal tissue[149-153] and delivered via the vasculature to the brain might have an important role in synaptic plasticity, memory function and mood regulation[151]. Adiponectin is an adipocyte-secreted protein that seems to have neuroprotective effects[152], in addition to its insulin-sensitizing, anti-inflammatory and antiatherogenic effects[152,154]. In mice, adiponectin was demonstrated to pass through the blood–brain barrier and was associated with increased neurogenesis and reduced depression-like behaviours[152]. Interestingly, discrepancies can exist between the plasma levels of adiponectin and levels in cerebrospinal fluid. For example, in humans, acute exercise increased plasma levels of adiponectin but decreased cerebrospinal fluid levels of adiponectin[155]. The mechanisms underlying the beneficial effects of exercise on brain structure and function remain an active area of investigation.

[H3] Muscle–brain crosstalk. Myokines seem to have an important role in hippocampal neurogenesis (animal model) and neurotrophin levels (animal model), and enhanced cognition and mood (animal model and humans) (**Supplementary Table 1**). For instance, in mice, increasing intrinsic irisin expression in neurons or increasing plasma levels of irisin elevates hippocampal *Bdnf* gene expression[153]. Irisin administration in a mouse model of Alzheimer disease improves synaptic plasticity and memory function[156,157]. In both animal models and humans, plasma levels of cathepsin B, a lysosomal thiol proteinase produced by muscle, is positively associated with hippocampus-dependent memory[146,158]. Studies in humans have shown that acute exercise does not clearly increase the plasma levels of cathepsin B[7], although chronic exercise does increase cathepsin B[146,158]. These same studies also showed that acute exercise increases plasma levels of BDNF[7], whereas chronic exercise does not increase BDNF[141,146] suggesting that investigation of local neuronal effects remains warranted.

***[H3] Liver–brain crosstalk*.** The liver secretes factors that are important for brain function. Kynurenine is a metabolite of the amino acid L-tryptophan and is primarily synthesized in the liver. In both mice and humans, chronic aerobic training increased muscle expression of kynurenine aminotransferase, which facilitates conversion of kynurenine into kynurenic acid, a metabolite that is unable to cross the blood–brain barrier. This shift in kynurenine metabolism was able to protect the brain from stress-induced depression[151].

Exchanging plasma from exercising aged mice to sedentary aged mice enhanced adult hippocampal neurogenesis and memory function[149]. Upon further investigation, plasma proteomic analysis led to the identification of a novel hepatokine, glycosylphosphatidylinositol–specific phospholipase D1 (GPLD1), which increased after exercise and correlated with improved cognitive function in aged mice. These findings are supported in humans, as concentrations of GPLD1 in blood were higher in active, healthy older adults (*n* = 20, >66 years old) than in their sedentary counterparts[149]. Investigations into the underlying mechanisms indicate that GPLD1 does not cross the blood–brain barrier[149]. Hence, the benefit of GPLD1[149] and other exerkines on brain structure and function might relate to peripheral effects, such as the complement signalling cascade[150,160], or coagulation[159]. Additional exercise studies are needed to better appreciate the exercise–liver–brain axis.

[H2] Bone

Exercise, especially resistance exercise, increases BMD[161]. Multiple mechanisms exist, although mechanical loading is considered a major factor[162]. Noted exercise-associated bone-derived factors affecting bone formation include TGFβ1 (Ref. [163]) and sclerostin[164]. Sclerostin inhibits bone formation[164,165] and blood levels of sclerostin are lower in highly active humans than in sedentary humans[164]. Emerging data demonstrates that crosstalk exists between bone and muscle, probably mediated by secretory factors[166]. Noted myokines affecting the bone

include apelin[167], myostatin[62], irisin[168], IL-6 (Ref. [169]), IL-7 (Ref. [61]) and BAIBA[60] (**Supplementary Table 1**).

[H1] Gaps and future opportunities

[H2] Gaps in exerkine science

Contentious questions remain that temper the enthusiasm regarding exerkines (**Box 1**). These controversies include the lack of consistency between the acute and chronic exercise response, discrepancies between humans and animal models of exercise and interpretation challenges due to variability in outcomes and sampling. These knowledge gaps set the stage for future opportunities in exerkine research.

Despite the acceleration in exerkine-related research since the identification of IL-6 as a myokine in 2000 (Ref. [16]), much remains to be done in the scientific areas of research, technology and therapeutic interventions (**Box 1**). Specifically, a critical need exists to move beyond the ‘skeletal muscle-centric’ view of exerkines and focus more on their roles in inter-organ communication, tissue regeneration, immune regulation, metabolic adaptation, cardiovascular fitness, psychological health and overall health across the lifespan. The vasculature and endothelium are emerging as a probable central facilitator, which enables systemic exerkines to exert their specific effects within the various local environments, as well as being a direct target and source of exerkines. Understanding the system-wide effects of exercise and the myriad of exercise-related improvements is essential for understanding resilience. Such knowledge will provide new translational research opportunities to develop novel, targeted interventions that increase physiological reserve, maintain and/or enhance resiliency and thus promote healthy ageing, as well as interventions that prevent and treat comorbidities and chronic disease.

Many more exerkines, their sources, targets and mechanisms remain to be discovered. In 2016, the MoTrPAC project was launched to uncover novel exerkines through deep omics

profiling of biomaterial from humans and rodents before and after acute exercise as well as chronic exercise training. As potential candidate transducers are uncovered, follow-up mechanistic studies will be required to delineate their function. In addition to molecular discoveries, substantial work remains to decipher the dosage and type of exercise needed to elicit positive health outcomes. Intervention studies are needed to investigate the effect of different types of exercise on resilience to various conditions, with guidance available from the NIH in designing resilience-based studies[170]. To address these knowledge gaps, detailed studies are needed to identify and validate the exerkine responses after exercise (acute, chronic or intermittent) exposure, including a detailed post-exercise response. Certainly, the demographics and phenotypes of the population will matter, as the response in healthy participants can vary greatly from participants with comorbidities. Further work in these areas will advance our understanding of ageing, health and disease prevention.

[H2] Opportunities for new technologies

Technology gaps also remain in exerkine research. One emerging area is the use of wearable technologies and devices to capture quantitative and dynamic phenotypes over long periods of time for healthy individuals as well as those with mild or severe diseases. For instance, wearable technology could provide valuable information regarding physical activity levels and exercise capacity during and following COVID-19 illness and recovery, adding to the description of post-acute sequelae of SARS-CoV-2 infection, which is under active investigation. Currently, non-invasive and minimally invasive devices have enabled the monitoring of many behavioural and physiological phenotypes, including heart rate and electrical activity in the heart (ECG), body temperature, physical activity and sedentary behaviour, peripheral blood oxygen saturation and blood concentrations of glucose. These devices enable the real-time monitoring of the effects of exercise in natural and controlled settings at an unprecedented level. Moreover, wearable technologies can be scaled to the analysis of over a million people and can enable

‘citizen science’ whereby individuals with devices can readily participate in studies. These measurements, when combined with molecular measurements such as exerkines, have the potential to greatly improve our understanding of exercise adaptations in large cohorts with deep phenotyping across a broad age range.

Although wearable devices are powerful, multiple challenges remain in data interpretation and analysis. These challenges include device accuracy as well as device standardization and a lack of readily available high-resolution data from the manufacturer. In addition, many wearable devices do not characterize the environmental context associated with data capture. For example, if a device does not sense any physical activity, one explanation could be the lack of movement by the wearer while another explanation could be device removal. A remaining challenge entails approval and regulation from entities such as the FDA before widespread use of wearables as therapeutic interventions.

In addition to wearables, the technology, analysis and approach for exerkine discovery needs to be further developed. Deep omics profiling (transcriptome, metabolome, proteome and lipidome) of human and animal-based exerkines is occurring in MoTrPAC; this effort is expected to reveal novel molecules and mechanisms involved in exercise by providing a more comprehensive view of the multi-omics landscape of exerkines to the research community. Extracellular vesicle analysis, especially of exosomes **[G]**, will be a critical component of MoTrPAC’s analysis owing to burgeoning interest in their role as carriers of molecular signals and drivers of inter-organ crosstalk.

[H2] Data reporting and data sharing

To promote comparison between studies and enhance translation, a crucial need exists for the establishment of community-wide standards for data reporting and data sharing. As an example, capturing physical activity and body composition (for example, adipose tissue mass) in the electronic health record will facilitate electronic health record data mining to examine clinical

outcomes outside of a structured clinical study. Cross-study comparisons of exercise studies will be facilitated by setting standards for a minimum metadata set, for consistent documentation and of covariates, such as time of day, diet or exercise exposure. Advances in computational modelling will accelerate our understanding of the physiological process of exercise and exerkine effects. Establishing a uniform knowledge base remains a critical 'next step' to driving this process.

[H2] Exerkines as therapeutics

The health benefits of exercise are well documented. However, not all individuals are able to benefit from exercise owing to physical limitations, such as paraplegia, or imposed limitations, such as quarantining during the current COVID-19 pandemic. Moreover, in humans, metabolic non-response or even adverse responses to exercise have been described.[26] As the role of exerkines and their biological effects are increasingly clarified, exerkines can be potentially harnessed to mimic the benefits of exercise in individuals who are limited in their exercise capacity or to counterbalance the metabolic non-response or adverse response to exercise. Although this 'exercise in a pill' is currently wishful thinking, it remains a tantalizing goal for future research directions.

[H1] Conclusions

Although exercise exerts many beneficial effects across multiple organ systems, our understanding of the mechanisms driving the benefits of exercise and the variability in these benefits remains rudimentary. Much of the initial exerkine research has been skeletal muscle-focused, however, contemporary research is now rapidly expanding to include non-skeletal muscle-based sources and targets for exerkines that contribute to maintaining and restoring health. Exerkines are increasingly recognized as critical mediators of exercise-related changes and health benefits, particularly in their role in inter-organ and systemic communication and

coordination. Yet, much remains to be done. To improve translation of results, the heterogeneity across studies needs to be minimized by reducing exposure variability and using standardized, consistent outcome measures. Large scale, structured studies will be key resources in providing a structured environment to pursue future exerkine-related questions. In summary, exerkines are a highly promising direction for future research initiatives, with high potential as biomarkers to predict outcomes, facilitate personalized exercise programmes to improve health, reduce disease and promote resilience across the lifespan.

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The authors contributed equally to all aspects of the article.

Competing interests

L.S.C. has received a Dexcom investigator initiated grant (product only). C.J.L. is a consultant for PAIhealth on their Personalized Activity Intelligence applications. A.P. is on the advisory board of Roche Diagnostics. M.P.S. is a cofounder and scientific advisory board member of Personalis, SensOmics, Qbio, January, Filtricine, Protos, NiMo, Mirvie. M.P.S. is an advisor for Genapsys. The other authors declare no competing interests.

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Supplementary information

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Key points

- Although the benefits of exercise are well acknowledged in enhancing health and treating disease, the molecular mechanisms underlying exercise-associated benefits remain ill-defined and are actively being investigated.
- ‘Exerkines’ encompass a broad variety of signalling moieties released in response to acute and/or chronic exercise that exert their effects through endocrine, paracrine and/or autocrine pathways.
- Exerkines can come in many forms, such as hormones, metabolites, proteins and nuclei acids; interest is increasing in moving beyond singular changes of specific factors to profiling exerkine alterations using ‘-omics’ platforms.
- There is burgeoning interest in the role of extracellular vesicles, which are membranous structures released from cells, serving as important carriers of molecular signals and drivers of inter-organ crosstalk related to exercise.
- Multiple organ systems, including the cardiometabolic system, nervous system and immune system, produce exerkines and are influenced by exerkines, which probably contributes to the pleiotropic and variable response to exercise.
- Emerging research on exerkines suggests multiple promising avenues for translational research and therapeutic modulation to capture exercise-associated benefits; enhanced rigour in experimental design will facilitate cross-comparison between studies.

Table 1: Examples of paracrine and autocrine effects of exerkines

Exerkine[a]	Source tissue	Affected tissue
Autocrine effects[b]		
12,13-diHOME	–	BAT[93,171]
Apelin	–	Muscle[96,98]
Adiponectin	–	WAT[154]
BDNF	–	Brain[172], muscle[173]
FGF21	–	WAT[57,174]
HSP72	–	Muscle[108,175]
IL-6	–	Muscle[15,55,121]
IL-7	–	Muscle[104]
IL-15	–	Muscle[176]
Lactate	–	Muscle[177]
LIF	–	Muscle[105]
Musclin (also known as osteocrin)	–	Muscle[44,178], bone[63,179], brain[180]
Myostatin	–	Muscle[45]
Nitric oxide	–	Endothelium[181]
Reactive oxygen species	–	Muscle[182]
SPARC	–	Muscle[64,183]
SDC4	–	Muscle[106]
TGFβ1	–	Muscle[184]
Paracrine effects		
Adiponectin	Adipose tissue	Muscle[59]
Angiopoietin 1	Vascular smooth muscle	Vasculature[48]
BAIBA	Muscle	WAT [56], bone[60]

BDNF	Muscle	Nerves[54]
Fractalkine	Muscle	Leukocytes[67]
FGF21	WAT	BAT[57]
IL-6	Muscle	WAT[15,122]
IL-7	Muscle	Bone[61]
IL-8	Muscle	Vasculature[49]
IL-13	Tissue-resident ILC2	Muscle[137]
IL-15	Muscle	WAT[58]
LIF	Nerves, immune cells	Muscle[185,186]
Musclin (also known as osteocrin)	Bone	Cartilage[63]
Myostatin	Muscle	Bone[62]
SPARC	Muscle	Extracellular matrix[64]
SDC4	Endothelium	Muscle[187]
TGFβ1	Muscle	Extracellular matrix[65]
TGFβ2	Adipose tissue	Muscle, BAT[43]
VEGF	Muscle	Endothelium[46,47]

[a]Although exerkine effects are commonly thought to be distant (endocrine) from the originating source tissue, exerkines also exert local effects within the originating tissue (autocrine) and neighboring tissues (paracrine). More details and relevant references are noted in **Supplementary Table 1**. [b]For exerkines with autocrine effects, the source tissue column contains '–', as the source tissue and affected tissue are the same. 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME), B-aminoisobutyric acid (BAIBA), Brown adipose tissue (BAT), Brain derived neurotrophic factor (BDNF), Fibroblast growth factor-21 (FGF21) , Heat Shock Protein-72 (HSP-72), Leukemia inhibitory factor (LIF), Secreted protein acidic and rich in cysteine (SPARC), Syndecan-4 (SDC4), Transforming growth factor beta 1 (TGFβ1) , Transforming growth factor beta 1 (TGFβ2) , Type 2 innate lymphoid cells (ILC2s), Vascular endothelial growth factor (VEGF), White adipose tissue (WAT).

Table 2: Common platforms for exerkine measurement

Platform	Commonly measured exerkines	Advantages	Disadvantages	Refs
Untargeted mass spectrometry	Lipidomics; metabolomics; proteomics	Profiles large number of molecules (>1,000 for metabolites and lipids; over 5,000 proteins); fairly inexpensive	Relative quantification rather than absolute quantification; bias toward abundant molecules; throughput lower than targeted assays	[31]
Targeted mass spectrometry	Lipidomics; metabolomics; proteomics	Accurate and absolute quantification; fast	Fewer molecules than untargeted assays	[31]
Immunoassays	Proteins (including cytokines) and metabolites	Accurate and absolute quantification; measures low abundance molecules	High throughput	[31]
RNA-seq	Transcripts; splicing isoforms	Comprehensive (>1,000 genes); accurate absolute quantification	Misses downstream events (e.g. posttranslational modifications)	[31,33]
Methyl-seq	DNA methylation	Measures stable epigenome changes	Expensive	[31,33]
ATAC-seq	Open chromatin	Measures chromatin epigenome changes	Easy to perform; high throughput	[31,33]

ATAC-seq, assay for transposase-accessible chromatin using sequencing; Methyl-seq, methylation sequencing; RNA-seq, RNA-sequencing

Table 3: Exerkines that affect the cardiometabolic system

Name	Species or model[a]	Main tissue of origin	Main target tissue	Effect[b]	Main biological action	Response to acute exercise bout[c]	Response to chronic training[c]	Highlighted studies
12,13-diHOME	H, A, C	BAT	BAT, skeletal muscle, heart	A, E	Increases fatty acid uptake	↑	↑	93 ,95 ,171
Adiponectin	H, A, C	WAT	Many tissues, including liver, muscle, heart	A, P, E	Enhances glucose and lipid utilization	↑	↑,↔	59,154 ,188
Angiopoietin I	H, A, C	Skeletal muscle	Vasculature	P	Enhances angiogenesis	↑ ,↔	↑,↓	48,69 ,70)
Angiopoietin-like protein 4	H, A	Liver,	WAT	E	Decreases lipoprotein lipase activity and enhance WAT lipolysis to increase plasma FFAs and triglycerides	,↑	↑,↔	39 ,69,80
Apelin	H, A, C	WAT, skeletal muscle	Skeletal muscle,	A, E	Enhances skeletal muscle mass and mitochondria	↑	↑,,↔	96 ,97,98
BAIBA	H, A, C	Skeletal muscle	WAT, Liver, β-cells	E, P	Enhances 'browning' of white adipocytes and β-oxidation in liver; attenuates insulin secretion from β-cells	↑	↑	56 ,114 ,189
Catecholamines	H	Adrenal	Skeletal muscle, WAT	E	Stimulates glycogenolysis; stimulates lipolysis of WAT	↑	↔	41
Fetuin-A	H	Liver	Skeletal muscle, pancreas	E	Impedes β-cell function; increases insulin resistance	↓,↔	↓	39,73 ,99 ,103,116
Fractalkine (also known as CX3CL1)	H, A, C	Skeletal muscle	Leukocytes, endothelium, myocytes, β-cells	P, E	Increases inflammatory, angiogenic, and chemotatic factors; regulates β-cell secretion.	↑	↔	67,71 ,72,115
FGF21	H, A	Many tissues, especially liver; also WAT	Many tissues, including heart and WAT	E, A, P	Augments fuel utilization (glucose, lipid); protects from apoptosis	↑	↔	57,73,74,92,174,190
Follistatin	H, A	Many tissues, especially liver	Skeletal muscle	E	Decreases serum levels of myostatin to enhance skeletal muscle growth; might affect glucose homeostasis	↑	↑	39,99,100,102,117,191
GDF15	H, A	Many sites	Many sites	E	Marker of stress response, promotes WAT lipolysis	↑	↑	81,192
HSP72	H, A	Many tissues, especially liver	Many sites	A, E	Maintains cellular homeostasis; protects cells from stress	↑	↑	108,175
IL-6	H, A, C	Primarily skeletal muscle	Many sites	A, P, E	Multiple effects: including enhancing WAT lipolysis; lipid oxidation; glucose homeostasis; anti-inflammatory response; skeletal muscle growth	↑	↓	15,16 ,30,79,82,120, 121 ,122
IL-7	H, A, C	Skeletal muscle	Skeletal muscle, bone	A, P	Regulates skeletal muscle development	↑	↔	104,193
IL-8	H, A, C	Skeletal muscle	Endothelium	P	Regulates tissue angiogenesis and blood flow	↑ , ↔	↔	49,51,75
IL-15	H, A, C	Many tissues, especially immune cells	Many tissues, especially immune cells	A, P, E	Regulates immune cell functioning and might reduce WAT mass ; improves glucose homeostasis; promotes	↑,↔	↔	58,101,176

		cells			skeletal muscle growth			
Irisin (also known as FNDC5)	H, A	Skeletal muscle	WAT, bone, β-cells, brain	E	Benefits primarily in animal models: increases fatty acid uptake; beiging of WAT; improves insulin secretion	↑	↓,↔	[85,118,168]
Lactate	H	Skeletal muscle	Many tissues, including central nervous system	A, E	Provides substrate for hepatic gluconeogenesis	↑	↔	[42,177]
METRNL	H, A, C	Many tissues, including WAT and skeletal muscle	Immune cells	E	Increases energy expenditure; improves glucose tolerance	↑	↑	[135,194,195]
Myonectin (CTRP15)	H, A	Skeletal muscle	Liver, WAT, heart	E	Promotes fatty acid uptake; might be cardio-protective	↑,↔	↔	[27,76,83,196]
Musclin (also known as osteocrin)	H, A, C	Skeletal muscle, bone, brain	Skeletal muscle, heart, vasculature, cartilage, brain	A, P, E	Regulates mitochondrial biogenesis and might exacerbate insulin resistance	↑	↓	[27,44,178,197]
Myostatin (also known as GDF8)	H, A, C	Many tissues, especially skeletal muscle and WAT	Many tissues, especially skeletal muscle and bone	A, P, E	Blunts skeletal muscle growth and glucose uptake	↑,↔	↔	[45,74,101,191]
SPARC	H, A, C	Many tissues	Many tissues	A, P and E	Regulates cell function and tissue remodelling	↑,↔	↔	[64,183]
SDC4	H	Many tissues	Immune system	A, P and E	Involved in cell-extracellular matrix cross talk, inflammation and skeletal muscle growth	↑	↑	[106,187,198,199]
TGFβ1	H, A, C	Many tissues	Many tissues especially immune cells	A, P, E	Chemotactic factor for immune cells; affects skeletal muscle growth	↑	↑	[65,66,184,200,201]
TGFβ2	H, A,C	Adipose tissue	Many tissues, especially muscle and immune cells	P, E	Promotes glucose and fatty acid metabolism; reduces inflammation	↑	↑	[43]
VEGF	H, A, C	Many tissues, especially skeletal muscle	Vascular endothelium	P, E	Promotes angiogenesis and exercise-induced neurogenesis	↑,↔	↑	[27,46,47]

[a]Relevant species or models are humans (H), animal (A) or cell (C). [b]Effects were autocrine (A), paracrine (P) or endocrine (E). [c]Arrows indicate: ↑, plasma levels increase; ↓, plasma levels decrease; ↔, plasma levels remain the same. 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME), B-aminoisobutyric acid (BAIBA), Brown adipose tissue (BAT), chemokine (C-X3-C motif) ligand 1 [CX3CL1], Complement C1q Tumor necrosis factor-Related Protein 15 (CTRP 15), Fibroblast growth factors-21 (FGF21) ,Fibronectin type III domain containing 5 (FNDC5), Growth and differentiation factor 15 (GDF15) , Growth and differentiation factor 8 (GDF8) , Heat Shock Protein-72 (HSP72) , IL-1 receptor antagonist (IL-1RA) , Interleukin (IL) 6 ,7, 8, 10, 13, 15 , meteorin-like (METRNL), Secreted protein acidic and cysteine rich (SPARC), Syndecan-4 (SDC4), Transforming growth factor beta 1 (TGFβ1), Transforming growth factor beta 2 (TGFβ2) ,Vascular endothelial growth factor (VEGF), White adipose tissue (WAT).

Figure 1: The systemic effects of exercise. a | Organs and tissues that can serve as source of exerkines and that are directly affected by exercise. **b |** Exercise results in profound health benefits, including reductions in the presence or severity of certain diseases, as well as increases to healthspan, longevity and resilience. T2DM, type 2 diabetes mellitus.

Figure 2: Exerkines that affect the cardiometabolic system. Exerkines released after exercise into the systemic circulation (see **Table 3** for tissue sources, detailed effects and relevant references) include proteins (blue lines), metabolites (yellow spheres) and extracellular vesicles (green spheres) to affect the cardiometabolic system. The effects are wide-ranging and systemic. In the cardiovascular system, exerkines enhance vascularization and angiogenesis, as well as improve blood pressure, endothelial function and overall fitness, resulting in cardioprotection. In adipose tissue, exerkines increase fatty acid uptake, enhancing lipolysis, thermogenesis and glucose metabolism. In the liver, exerkines enhance glucose metabolism and fatty acid uptake. In skeletal muscle, exerkines enhance muscle formation, maintenance and repair, glucose uptake, lipid oxidation, mitochondrial biogenesis and muscle capillarization. In the pancreas, exerkines enhance cell viability and influence insulin secretion. Commonly described exerkines are noted. 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME), B-aminoisobutyric acid (BAIBA), Growth and differentiation factor 15 (GDF15) , Heat Shock Protein-72 (HSP72), meteorin-like (METRNL), Secreted protein acidic and cysteine rich (SPARC), Syndecan-4 (SDC4), Transforming growth factor beta 1 (TGFβ2) , Vascular endothelial growth factor (VEGF).

Figure 3: Effects of exercise on the immune system. Exercise induces lipid oxidation, mitochondrial biogenesis and local injury, which stimulates the exerkine release into the circulation to influence the immune system. See **Supplemental Table 1** for detailed effects and relevant references. These include proteins (blue lines), metabolites (yellow spheres) and extracellular vesicles (green spheres), which have a multitude of effects on the immune system (generically represented by a monocyte). Acutely, exercise increases cytokines such as circulating levels of transforming growth factor β1 (TGFβ1) and IL-6 relative to the resting state. This change results in acute inflammation, characterized by increases in tumour necrosis factor (TNF) and IL-6. Once the acute exercise-induced effects have diminished, a subsequent increase of anti-inflammatory cytokines (such as IL-10 and IL-1 receptor antagonist (IL-1RA)) occurs, to respond to the acute inflammatory response. Chronic training is associated with a reduction in systemic and tissue inflammation, as characterized by lower circulating levels of TNF and IL-6 in the resting state, relative to sedentary individuals. Reduced insulin resistance and tumour growth has been attributed to the effects of chronic training on decreasing systemic and/or tissue inflammation.

Figure 4: Effects of exercise on the nervous system. Exercise stimulates the production of exerkines from tissues, such as skeletal muscle, adipose tissue or the liver, to affect the nervous system. See **Supplemental Table 1** for detailed effects and relevant references. These exerkines are released into the circulation and include proteins (blue lines), metabolites (yellow spheres) and extracellular vesicles (green spheres), which have a multitude of purported effects on the nervous system. These effects include increasing production of brain-derived neurotrophic factor (BDNF), enhancing neurogenesis (even in adults), cognition, mood and synaptic plasticity. The extent to which exerkines cross the blood–brain barrier to exert their effects remain unknown, symbolized by the question mark. There is uncertainty with GDF15, as marked by a (?), as pharmacological GDF15 inhibits appetite and reduces activity, whereas physiological induction of GDF15 by exercise does not[202]. Commonly described exerkines are noted. Fibroblast growth factors 21 (FGF21), Growth and differentiation factor 15 (GDF15), Glycosylphosphatidylinositol–specific phospholipase D1 (GPLD1).

Box 1: Contentious questions regarding exerkines

Overenthusiasm about the potential of exerkines needs to be tempered, as controversies remain.

[bH1] Inconsistency between the acute and chronic exerkine response

As demonstrated in **Supplementary Table 1**, the acute exerkine response does not necessarily parallel and could even contradict the chronic exercise response, as exemplified by brain-derived neurotropic factor (BDNF), IL-6, irisin and musclin. Hence, the question arises whether the discrepancy reflects the difference in mechanism between acute versus chronic perturbation, or more pragmatically, remains unrelated to the observed exercise-induced changes.

[bH1] Inconsistency between animal and human studies

As demonstrated in **Supplementary Table 1**, the exerkine response in animals does not necessarily parallel the exerkine response in humans, as exemplified by angiopoietin, BDNF, follistatin, IL-7, IL-8, IL-10, irisin, leukemia inhibitory factor, myonectin, musculin, myostatin and secreted protein acidic and rich in cysteine (SPARC). Hence, the question arises whether the excitement generated from discoveries in animal models will translate to specific human populations or more pragmatically, whether animal findings remain unrelated to observed changes in humans.

[bH1] Variability of outcomes and sampling, which hinder interpretation

The literature is replete with studies reporting variable, if not conflicting, benefits from exercise, commonly attributed to differences in selected populations, exercise type, exercise intensity and exercise duration[142,143,203]. However, even when an exercise exposure is fixed, as exemplified by a clinical trial setting, the cardiovascular response can be ostensibly disassociated from the

metabolic response[25,26,204]. Hence, the question arises whether these discrepancies can be explained by differences in the exerkine response. If exerkines might explain the observed findings, even more questions arise regarding sampling relative to exercise exposure (during versus after), site (tissue versus blood) and context (fasted or fed,[22] or relative to circadian rhythm[21,24]) Hence, the question arises about the translational relevance of the exerkine literature to date, given the inconsistency of observed exercise effects and highly variable sampling protocols.

Glossary

Resilience: Resilience is the ability of the body to resist, adapt to, recover or grow in response to stressors.

Exerkines: Exerkines encompass a broad variety of signalling moieties that are released in response to acute and/or chronic exercise that exert their effects through endocrine, paracrine and/or autocrine pathways.

Acute exercise: Acute exercise is typically considered a single episode of exercise (often resistant or aerobic exercise) that is completed during one visit.

Chronic exercise: Chronic exercise is typically described as multiple exercise episodes (often resistant or aerobic exercise) performed over the course of weeks to months.

High-intensity interval training: High intensity interval training is a form of exercise training characterized by bursts of high intensity activity followed by less intense recovery periods.

MicroRNA: MicroRNAs are non-protein coding RNA molecules that are regulated in a transcriptional or post-transcriptional fashion to affect mRNA transcription and/or degradation.

Exosomes: Exosomes are a type of extracellular vesicle released by parent cells, which contain RNAs, proteins and lipids, to facilitate cross-talk between tissues.

Exerkines are signalling moieties that are released in response to acute and/or chronic exercise that exert their effects through endocrine, paracrine and/or autocrine pathways. This Review summarizes the importance and current state of exerkine research, prevailing challenges and future directions.