

## REVIEW ARTICLE

# Exercise intensity matters: A review on evaluating the effects of aerobic exercise intensity on muscle-derived neuroprotective myokines

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## Abstract

Exercise as a medical intervention is effective to help prevent and manage many chronic and complex diseases, including dementia. There is evidence to suggest that regular aerobic exercise protects against age-related brain atrophy and reduces the risk of cognitive decline. The mechanisms by which exercise infers a neuroprotective effect remain to be established but may be related to a maintenance of brain volume and neuronal survival, improved cerebrovascular density and function, and/or increased synaptic plasticity. In addition, there is growing evidence to suggest the beneficial effects of exercise on brain health and cognitive function are, at least in part, mediated by factors released by skeletal muscle during contraction. The fact that the brain responds to exercise suggests that muscle-derived peripheral factors, or “myokines,” may play a key role in muscle–brain crosstalk and exercise neuroprotection. However, the most effective “dose” of aerobic exercise to promote beneficial changes in these myokine pathways is currently unknown. Specifically, most of the evidence to date is from studies that have used moderate-intensity exercise, and research investigating the merit of high-intensity exercise is scarce. Considering the well-established role of high-intensity interval training in protecting against numerous medical conditions, more research is needed to identify the most effective “dose” of exercise to improve the beneficial effects of these myokines.

## KEYWORDS

dementia, exercise intensity, high-intensity exercise training, moderate-intensity exercise training, myokines, neuroprotection

## Highlights

- **Neuroprotection through exercise:** Regular aerobic exercise mitigates age-related brain atrophy and cognitive decline via multiple mechanisms, including brain volume maintenance, improved cerebrovascular function, and synaptic plasticity.

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- **Myokines as mediators:** Muscle-derived factors (myokines) play a crucial role in muscle–brain crosstalk, significantly contributing to the neuroprotective effects of exercise.
- **Intensity matters:** The review underscores the necessity to define and study exercise intensity, revealing high-intensity exercise may be as effective, if not more, in promoting neuroprotective myokine levels compared to moderate-intensity exercise.
- **Future research directions:** This review emphasizes the need for well-controlled studies to explore the optimal exercise dose for enhancing myokine pathways and their implications for neurodegenerative disease prevention.

## 1 | CONTEXT

Aging is a complex biological process involving progressive physiological decline and increased disease susceptibility.<sup>1</sup> Molecular, cellular, and systemic modifications disrupt tissue equilibrium, leading to age-related disorders that include cardio-metabolic disease, cancer, and neurodegenerative diseases such as dementia.<sup>1</sup> In addition to the profound biological impact, aging poses significant financial and social implications for societies worldwide.<sup>2</sup> The rising life expectancy contributes to a higher proportion of older individuals in the population, leading to an amplified need for health-care services, long-term care facilities, and social support systems. This demographic change strains health-care resources and pension systems, necessitating innovative approaches to tackle the many challenges posed by an aging society.<sup>2</sup>

One of the greatest challenges associated with improved life expectancy is the increased incidence of dementia—a group of symptoms that impair cognitive functions and the ability to perform daily activities.<sup>3</sup> There are currently  $\approx$  55 million people worldwide diagnosed with dementia,<sup>3</sup> and every year there are nearly 10 million new cases.<sup>3</sup> Dementia is a progressive and degenerative disorder with no cure, which highlights the importance of preventative strategies for risk reduction.<sup>4</sup> Strategies to alter modifiable risk factors, like smoking, high blood pressure, high cholesterol, poor nutrition, and physical inactivity, which all contribute to dementia risk, have crucial roles in reducing the prevalence of dementia.<sup>4</sup>

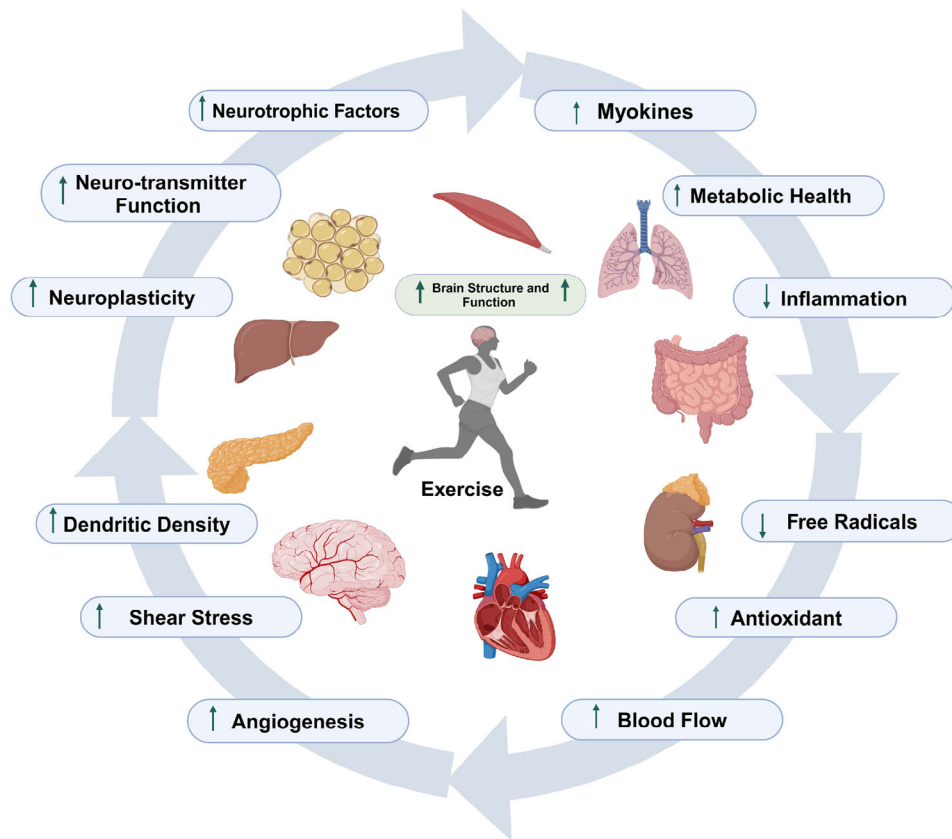
Physical activity involves skeletal muscle contractions that surpass baseline energy expenditure, while exercise is a deliberate, organized, and repetitive subset of physical activity targeting enhanced physical fitness or specific health benefits. Physical inactivity is a recognized risk factor for neurological disorders like dementia.<sup>4</sup> Conversely, increased physical activity levels have been linked with improvements in both brain volume and cognitive health.<sup>5</sup> Exercise is also recognized as a potential non-pharmaceutical intervention for managing neurodegenerative pathology,<sup>4</sup> with long-term interventions showing promise in reducing age-related cognitive decline in both healthy older adults and individuals with mild cognitive impairment.<sup>5</sup>

Exercise has a profound effect on the structure and function of the brain. It has been observed to improve neuroplasticity (the brain's

capacity to adapt and change in response to stimuli), improve neurovascular coupling (the close relationship between neuronal activity and blood flow regulation in the brain), increase dendritic density, increase cerebrovascular density and function,<sup>6</sup> as well as promote adult hippocampal neurogenesis (AHN, the process of generating new neurons in the hippocampus), among other adaptations (Figure 1). The mechanisms underlying the beneficial effects of exercise on brain health are multifaceted, and the regulation of neurotrophic factors—vital peptides facilitating neuronal growth, survival, and differentiation (the process by which undifferentiated neural cells undergo specialization and maturation to become functional neurons with distinct structures and functions).<sup>6</sup> In addition, there is growing evidence to suggest that the beneficial effects of exercise on brain health and cognitive function are, at least in part, mediated by muscle-derived factors (myokines) that exert endocrine effects on the brain.<sup>7</sup>

During exercise, skeletal muscle cells secrete myokines, which are cytokines and peptides with autocrine, paracrine, and endocrine effects.<sup>7</sup> Specifically, the myokines fibronectin type III domain-containing protein 5 (FNDC5), irisin, cathepsin B (CTSB), vascular endothelial growth factor (VEGF), as well as metabolites in the kynurenine pathway (a metabolic pathway responsible for the breakdown of the tryptophan (TRP)), are all upregulated during exercise<sup>7</sup> and all indirectly or directly upregulate brain-derived neurotrophic factor (BDNF).<sup>8</sup> BDNF is a trophic factor expressed in multiple areas of the brain, but predominately in the hippocampus,<sup>9</sup> with a central role in myokine–brain interactions stemming from its multi-functionality. Being a pivotal neurotrophin, BDNF not only supports neurogenesis, new neuron maturation, and integration, enhancing brain flexibility and cognition,<sup>9</sup> but also plays a significant role in the intricate mechanisms of AHN. Additionally, BDNF aids synapse formation and plasticity (Figure 2), which is vital for efficient neural communication, learning, and memory.<sup>9</sup> Key myokines exert neuroprotective effects via the upregulation of BDNF.<sup>8</sup> As such, the release of myokines during muscle contraction may play a key role in the effectiveness of exercise in preventing cognitive impairment and neurodegeneration.<sup>10</sup>

Not all exercise is equal; exercise intensity plays a crucial role in determining the physiological and metabolic responses to exercise, which in turn have significant implications for health outcomes.<sup>11</sup>



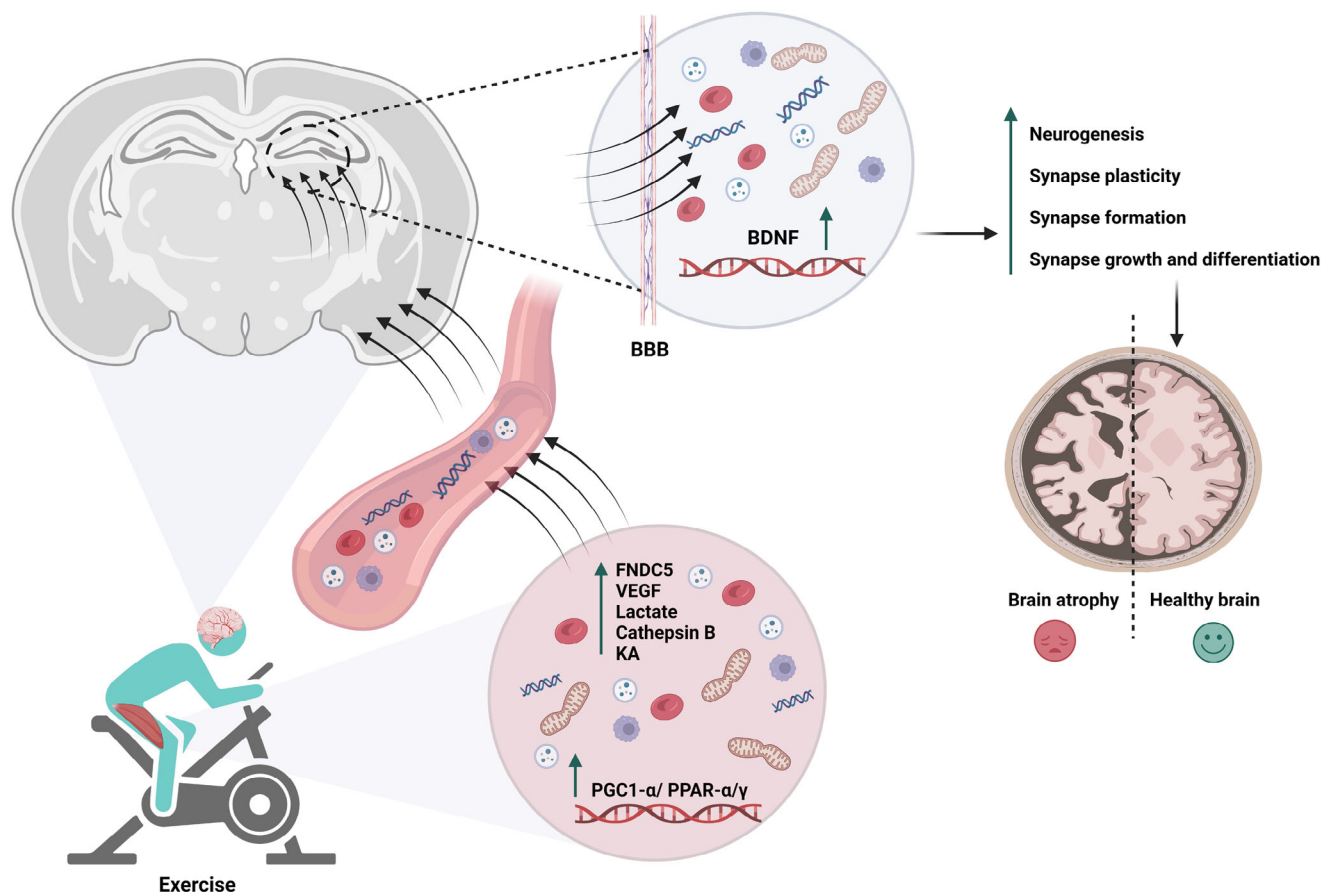
**FIGURE 1** Exercise and brain health mechanisms. Illustrating the hypothesised mechanisms through which exercise training can impact brain structure and function through peripheral and systematic changes. Generated by N.Z. in Biorender.

High-intensity interval training (HIIT;  $\approx$  85%–100% of the maximal power [ $\dot{V}_{\text{max}}$ ])<sup>12</sup> significantly improves cardiovascular and metabolic health by enhancing  $\dot{V}_{\text{O}_{2\text{max}}}$  (the maximal level of oxygen consumption, which is not increased with further increases in exercise intensity), blood pressure, insulin sensitivity, and lipid profiles through repeated bouts of intense activity with short recovery periods.<sup>12</sup> Some studies show that HIIT is superior to traditional moderate-intensity training improving  $\dot{V}_{\text{O}_{2\text{max}}}$ .<sup>13</sup> The greater training-induced increases in  $\dot{V}_{\text{O}_{2\text{max}}}$  with HIIT can be attributed to its unique physiological impacts, particularly enhancements in cardiovascular function, such as optimizing heart pumping capacity and improving systemic vascular function. Moreover, HIIT is associated with favorable alterations in blood flow dynamics, which may be attributed to its effects on strain and shear stress within the vasculature.<sup>11</sup> Adaptations in systemic vascular function are also connected to enhancements in brain vascular function. Improved  $\dot{V}_{\text{O}_{2\text{max}}}$ , particularly with HIIT compared to moderate-intensity continuous training (MICT), is linked to elevated cerebral perfusion and cerebrovascular reactivity throughout the human lifespan.<sup>11</sup> Overall, these findings indicate that high-intensity exercise is an effective approach for improving cardiovascular and metabolic health across varying ages, health statuses, and fitness levels.

Despite the fact that exercise intensity matters, few studies have focused on the effects of exercise intensity on myokines implicated in neuroprotection and on markers of neuroprotection (e.g., AHN).

High-intensity exercise will increase muscle and blood lactate concentrations to a greater extent than moderate-intensity exercise, which may prove a more effective stimulus for lactate (and other myokine)-dependent increases in brain BDNF.<sup>8</sup> In support of this, it was recently reported that high-intensity training was superior to moderate-intensity training for increasing muscle FNDC5 and hippocampal plasticity markers (VEGF, peroxisome proliferator-activated receptor gamma coactivator 1-alpha [PGC-1 $\alpha$ ], and tyrosine kinase B receptor [TrkB]) in rats.<sup>14</sup> In addition, considering that training intensity has been reported to modulate PGC-1 $\alpha$  protein content,<sup>15</sup> and kynurenine aminotransferases (KAT; enzymes that catalyze the synthesis of kynurenine acid [KA], a neuroprotective metabolite) are known to be activated by PGC-1 $\alpha$ , it is possible that high-intensity exercise is more effective than moderate-intensity exercise for increasing the content of KATs in skeletal muscle, and, therefore, for reducing the neurotoxic effects of increased brain kynurenine availability. This review will critically analyze the effects of exercise training intensity (i.e., high intensity vs. moderate intensity) on key neuroprotective myokine pathways (FNDC5, irisin, CTSB, VEGF, kynurenine pathway metabolites), as well as the effects of training intensity on a key marker of exercise neuroprotection, AHN.

The myokines included in this review have been shown, as previously mentioned, to enhance neurogenesis, synaptic plasticity, and cognitive function in response to both acute and chronic exercise



**FIGURE 2** Myokine effects on BDNF and ultimately on brain health. Several myokines, including FND5, irisin, CTSB, and VEGF are released from the skeletal muscle during exercise and contribute to the neuroprotective effects of exercise. These myokines act indirectly on brain health by crossing the BBB and enhancing BDNF expression in the brain. BDNF then improves neurogenesis, synapse plasticity, formation, growth, and differentiation. The intricate muscle–brain endocrine loop suggests a central role of BDNF in mediating the effects of myokines on brain function. Generated by N.Z. in Biorender. BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor. CTSB, cathepsin B; FND5, fibronectin type III domain-containing protein 5; KA, kynurenic acid; PGC-1 $\alpha$ , proliferator-activated receptor gamma coactivator 1-alpha; PPAR $\alpha/\delta$ , peroxisome proliferator-activated receptor; VEGF, vascular endothelial growth factor.

interventions. Notably, evidence suggests that exercise-induced modulation of myokines plays a critical role in mediating brain health, with potential implications for reducing the risk or progression of neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. These findings provide a foundation for investigating the role of exercise intensity as a key determinant in regulating myokine-mediated brain outcomes.

The following sections will (1) provide context for the principles of exercise prescription, with a particular focus on exercise intensity; (2) provide an overview of the role of key myokine pathways and outcomes associated with exercise neuroprotection; and (3) investigate the role of exercise intensity on the regulation of each of these factors in both human and animal models.

## 2 | PRINCIPLES OF EXERCISE PRESCRIPTION

When prescribing a training intervention, several variables must be considered, such as exercise intensity, duration, and frequency; the

overall length of the training intervention; and total training volume (the product of the session duration by the exercise intensity by the number of sessions). While exercise duration and frequency are unambiguous terms, the definition of exercise intensity, one of the key variables of a training intervention, is surprisingly complex and variable among studies. Indeed, concerns have been raised about the inconsistent use of terminology associated with determining exercise intensity in different studies.<sup>16</sup> This inconsistent determination of exercise intensities is also apparent in some of the studies that have investigated myokines and associated molecular pathways (Tables S1–S6 in supporting information). It is challenging to effectively harness the results of individual studies without establishing uniform terminology and common characteristics for the different exercise terminology used.

Consistent with a recent expert statement<sup>17</sup> calling on the standardization of exercise intensity terminology, this review will use three cardiorespiratory exercise intensity descriptors (low, moderate, and high), based on the direct assessment of metabolic thresholds and  $\dot{V}_{O_{2max}}$  or velocity ( $V_{max}$ ) achieved at  $\dot{V}_{O_{2max}}$  during an incremental

exercise test, to classify the intensity used in each study. Specifically, “low” refers to exercise intensities below the first metabolic threshold (the first detectable change in metabolism), “moderate” refers to exercise intensities above the first metabolic threshold but below the second metabolic threshold (the second detectable change in metabolism), and “high” refers to exercise intensities above the second metabolic threshold but below  $\dot{W}_{\max}$  or  $V_{\max}$ . Exercise below the first metabolic threshold is also commonly described as low-intensity training,<sup>16</sup> exercise performed at an intensity greater than the first metabolic threshold but less than the second metabolic threshold typically includes MICT,<sup>16</sup> exercise greater than the second metabolic threshold but less than  $\dot{V}O_{2\max}$  commonly includes HIIT,<sup>16</sup> while efforts performed at intensities equal to or greater than the work rate associated with the attainment of  $\dot{V}O_{2\max}$  include sprint interval training (SIT).<sup>16</sup>

Despite providing a more precise measure of relative exercise intensity, few studies investigating myokines and associated molecular pathways have determined exercise intensity zones via threshold-based assessments. Instead, exercise intensity is more often described/prescribed as a percentage of a predetermined maximal anchor, including percentages of  $\dot{V}O_{2\max}$ , peak oxygen uptake ( $\dot{V}O_{2\text{peak}}$ , the highest value of oxygen consumption attained during an incremental exercise test), maximum heart rate ( $HR_{\max}$ ),  $\dot{W}_{\max}$ , or  $V_{\max}$ . Despite their continued use within research, a major limitation of this approach is the lack of consistency in the metabolic stress elicited between individuals. For example, exercising at 80% of  $HR_{\max}$  was reported to elicit metabolic stress above the first metabolic threshold for only half of the participants prescribed this intensity, whereas the other half were below the first metabolic threshold.<sup>18</sup> As such, these approaches can result in the misclassification of exercise intensity categories for different individuals, ultimately inducing varying metabolic stresses.

It is important to acknowledge that most studies included in this review did not strictly conform to our predefined criteria for exercise intensity, making comparisons between studies that use different criteria and classifications challenging. However, we have endeavored to classify/re-classify each study based on the intensity descriptors proposed in Jamnick et al.<sup>16</sup> For studies that used alternative methods to prescribe exercise intensity (e.g., a percentage of a maximal anchor), the intensity was classified based on the three cardiorespiratory exercise intensity descriptors described in Table 1.<sup>16</sup>

Animal models are crucial for studying exercise physiology and diseases but must be carefully chosen and standardized tests are vital for fair comparisons. Implementing uniform guidelines is essential to boost research reproducibility, broaden the relevance of findings, and advance targeted therapies for different health conditions. Establishing exercise intensity standards in animal models, particularly rodents, is challenging due to species variability, as well as individual fitness and equipment differences. For more information regarding guidelines for animal exercise and training protocols, please refer to the review by Poole et al.<sup>19</sup>

In studies involving rodents, the classification of exercise intensity can be determined using various parameters, including treadmill

speed, heart rate, and  $\dot{V}O_2$ , among others. However, these values may fluctuate depending on variations in strain, age, and fitness level of the animals;<sup>19</sup> as such, there is no consensus on criteria to define exercise intensity in rodents. For the purpose of this review, and considering heart rate and  $\dot{V}O_2$  increase linearly with running duration or speed in rats and other species during exercise,<sup>19</sup> we have split exercise intensity for rodents into three categories based on treadmill speed and/or  $\dot{V}O_{2\max}$ .<sup>19</sup> These three categories are low intensity (10–15 m/minutes for mice and 12–20 m/minutes for rats, and/or 50%–60% of  $\dot{V}O_{2\max}$ ), moderate intensity (15–20 m/minutes for mice and 20–25 m/minutes for rats, and/or 60%–70% of  $\dot{V}O_{2\max}$ ), and high intensity (above 20 m/minutes for mice and 25 m/minutes for rats, and/or 70%–80% or more of  $\dot{V}O_{2\max}$ ).<sup>19</sup> Furthermore, based on Poole et al.,<sup>19</sup> we categorized free wheel running as low- to moderate-intensity exercise, unsuitable for maximal exercise studies due to its brief, erratic nature, typically lasting less than 2 minutes. While promoting cardiovascular fitness in rodents, it lacks precision in controlling both duration and intensity.<sup>19</sup>

### 3 | BDNF

#### 3.1 | The role of BDNF

BDNF is a trophic factor expressed in multiple areas of the brain but is predominately expressed in the hippocampus. BDNF has different biologically active isoforms (e.g., pre-pro-BDNF, pro-BDNF, and mature BDNF) that interact with many different receptors (e.g., sortilin, neurotrophin receptor P75 [p75NTR], TrkB),<sup>9</sup> meaning it is involved in a wide spectrum of neurophysiological pathways.<sup>7,9</sup> The binding of BDNF to these receptors initiates signaling pathways that support the development and upkeep of new neural connections, as well as the preservation of existing ones, crucial aspects of AHN.<sup>9</sup> Once BDNF attaches to TrkB, it results in the auto-phosphorylation of TrkB, which then becomes a binding site for proteins initiating some of the main intracellular signal cascades. These cascades include phosphoinositide 3-kinase (PI3K)/AKT, phosphoinositide phospholipase C- $\gamma$  (PLC $\gamma$ ), mitogen-activated protein kinase (MAPK), and nucleotide guanosine triphosphate (GTP)ases (GTPases), which regulate gene expression and protein synthesis crucial for neural connection formation, maintenance, and synaptic plasticity.<sup>9</sup>

BDNF has a central role in diverse neurobiological phenomena, notably neurogenesis (a dynamic process of creation, maturation, migration, and integration of new neurons) and synapse formation (a critical mechanism essential for efficient neuronal communication and neural circuitry development).<sup>9</sup> Additionally, BDNF actively regulates neuroplasticity, learning, and memory.<sup>9</sup> Furthermore, it is involved in the growth and differentiation of newly generated hippocampal neurons<sup>9</sup> (Figure 3).

Finally, it is noteworthy that BDNF can cross the blood–brain barrier (BBB) bidirectionally, but the mechanisms and the extent to which peripheral BDNF influences central nervous system levels are not fully understood. Evidence suggests that circulating BDNF might partially



**TABLE 1** Exercise training intensity categories in humans and rodents.

	Human	Rodents
<b>Low intensity</b>	<ul style="list-style-type: none"> <li>• &lt; the first lactate threshold</li> <li>• &lt; 2 mmol/L blood lactate level</li> <li>• &lt; 75% <math>\dot{V}O_{2max}</math></li> <li>• &lt; 80% <math>HR_{max}</math></li> <li>• &lt; Gas exchange threshold</li> <li>• &lt; Ventilatory threshold</li> </ul>	<ul style="list-style-type: none"> <li>• 10–15 m/minutes for mice</li> <li>• 12–20 m/minutes for rats</li> <li>• 50%–60% of <math>\dot{V}O_{2max}</math></li> </ul>
<b>Moderate intensity</b>	<ul style="list-style-type: none"> <li>• 2–4 mmol/L blood lactate level</li> <li>• 75%–85% <math>\dot{V}O_{2max}</math></li> <li>• 80%–90% <math>HR_{max}</math></li> <li>• Gas exchange threshold /ventilatory threshold/lactate threshold &lt; respiratory compensation point/ maximal lactate steady state/ second lactate threshold</li> </ul>	<ul style="list-style-type: none"> <li>• 15–20 m/minutes for mice</li> <li>• 20–25 m/minutes for rats</li> <li>• 60%–70% of <math>\dot{V}O_{2max}</math></li> </ul>
<b>High intensity</b>	<ul style="list-style-type: none"> <li>• &gt; 4 mmol/L blood lactate level</li> <li>• &gt; 85% <math>\dot{V}O_{2max}</math></li> <li>• &gt; 90% <math>HR_{max}</math></li> <li>• &gt; Respiratory compensation point/ maximal lactate steady state/ second lactate threshold</li> </ul>	<ul style="list-style-type: none"> <li>• Above 20 m/minutes for mice</li> <li>• Above 25 m/minutes for rats</li> <li>• 70% or more of <math>\dot{V}O_{2max}</math></li> </ul>

Note: Lactate threshold is the point at which lactate is produced and accumulates in the blood at a faster rate than it can be removed. Abbreviations:  $HR_{max}$ , maximum heart rate;  $\dot{V}O_{2max}$ , maximal level of oxygen consumption

reflect brain-derived BDNF, although platelets, muscle, and other tissues also contribute significantly to BDNF levels. Consequently, serum BDNF levels might not exclusively represent BDNF activity within the hippocampus or other brain regions.

### 3.2 | Exercise intensity and BDNF

There are a few systematic/literature reviews investigating the effects of exercise intensity on BDNF. Feter et al.<sup>20</sup> reported that exercise intensity (when above at least 65% of  $\dot{V}O_{2max}$ ) has a linear association with BDNF changes in the plasma or serum of human adults (> 18 years), and this increase was independent of age group and health status. Their review included experimental studies with any model of exercise (e.g., continuous aerobic, resistance, and HIIT). It is noteworthy that elevated blood BDNF levels post-exercise may not solely mirror brain BDNF changes, as serum BDNF originates from diverse sources, including the brain, muscles, and other tissues. In addition, a systematic review by Fernández-Rodríguez et al.<sup>21</sup> reported that a single session of high-intensity interval exercise (i.e., 75%  $HR_{max}$  or  $\dot{V}O_{2max}$ ) induced a larger increase in serum or plasma BDNF compared to both inactivity and low-intensity (no details were provided) interval exercise; however, there were no observed differences between high-intensity and moderate-intensity (no details were provided) interval exercise. Notably, the categorization of 75%  $HR_{max}$  or  $\dot{V}O_{2max}$  as “high,” which does not correspond with our definition of “high” (> 85%  $\dot{V}O_{2max}$ , Table 1), may have contributed to this outcome and suggests a potential misclassification of exercise intensity.

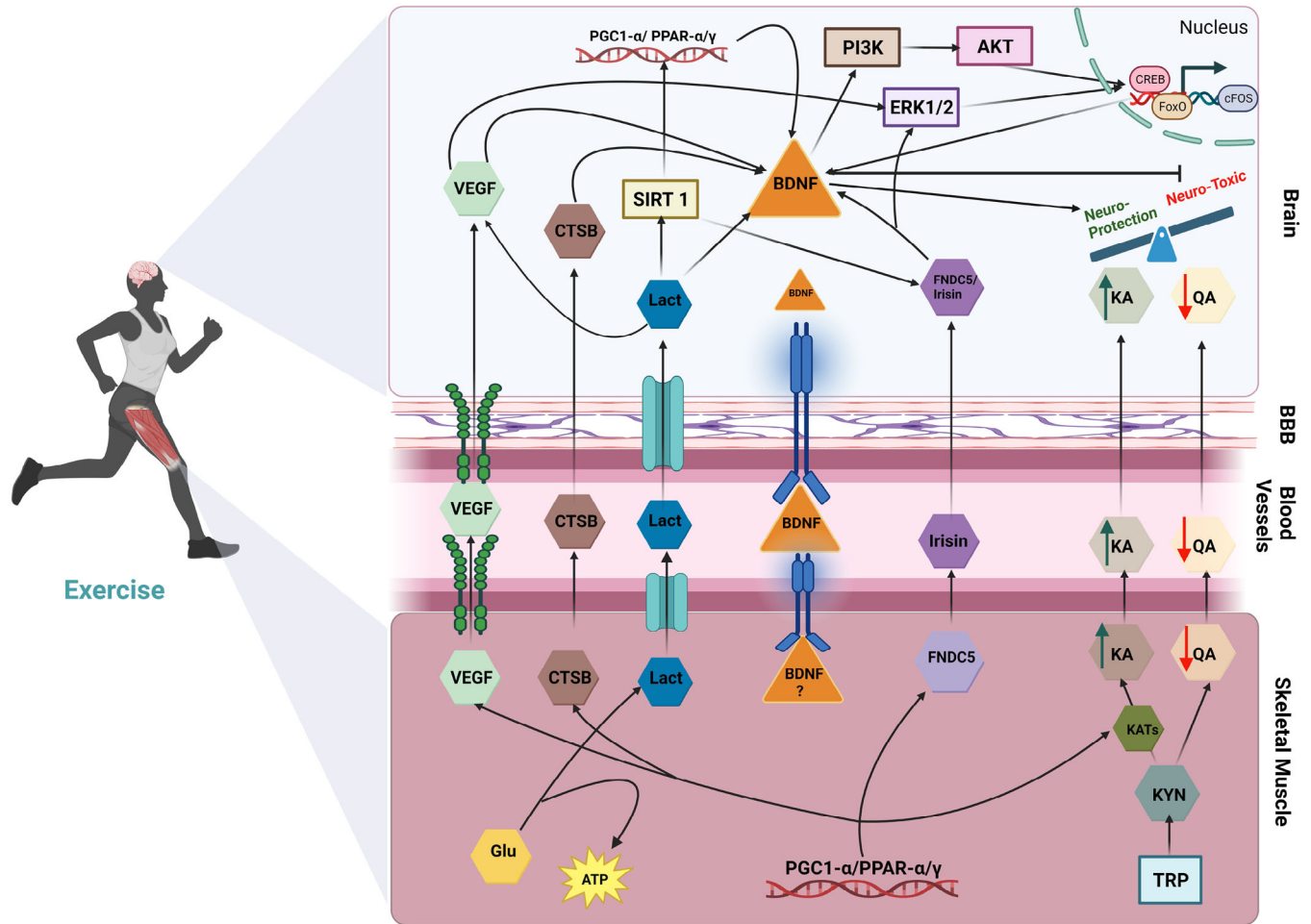
Exercise-induced alterations in the concentration of BDNF have also been investigated in rodent brain tissue. For example, De Almeida et al.<sup>22</sup> investigated the impact of exercise intensity on hippocam-

pal BDNF levels in developing rats (rats were divided into different age groups: P21–P30, pre-puberty; P31–P40, juvenile; P41–P50, adolescent; P51–P60, adult). Specifically, rats (at a developmental stage of P41–P50) who performed moderate-intensity exercise (30 minutes daily running at 20 m/minute—classified as per Table 1, for 10 days) exhibited significantly higher concentrations of BDNF in the hippocampus compared to both low-intensity exercise (16 m/minute) and control (no-exercise) rats. In addition, there were no observed differences in BDNF levels in other age groups.<sup>22</sup> Despite the potential misclassification of intensity, an increase in exercise intensity resulted in increased BDNF that might imply a significant influence of exercise intensity on the regulation of BDNF. In further support of this, exercise intensity has also been observed to regulate a number of factors upstream of BDNF (i.e., neuroprotective myokines) across multiple tissues, including muscle, blood, and the brain, as discussed below.

## 4 | FIBRONECTIN TYPE III DOMAIN-CONTAINING PROTEIN/IRISIN

### 4.1 | The role of fibronectin type III domain-containing protein/irisin

Fibronectin type III domain-containing protein is released primarily from contracting skeletal muscle fibers.<sup>7</sup> Peroxisome PGC-1 $\alpha$ -induced cleavage of FNDC5 produces irisin, a 12-kD membrane protein.<sup>23</sup> The cleavage of FNDC5 to irisin occurs at the extracellular domain of the protein within the cell membrane of muscle cells.<sup>23</sup> Irisin is secreted by muscle during exercise, crosses the BBB, and has been implicated in promoting BDNF expression.<sup>8</sup> Irisin-induced activation of BDNF in the brain, specifically in the hippocampus, has a wide



**FIGURE 3** Exercise and neuroprotective myokines. Exercise-induced increases in lactate can cross the BBB via Monocarboxylate transporters and stimulate BDNF expression and TrkB signalling in the hippocampus, potentially mediated by lactate-induced activation of SIRT1 and subsequent induction of peroxisome PGC-1α, FND5, and BDNF. Additionally, VEGF promotes neurogenesis and cerebral angiogenesis in the brain through effecting on ERK1/2 or directly on BDNF. Glycosylation and cleavage of FND5 lead to the production of irisin, which can cross the BBB and exert its effects on brain tissue, including BDNF expression in the hippocampus. On the other hand, kynurenine pathway metabolites such as KA are affected by peroxisome PGC-1α to improve neuroprotection/neurotoxic ration. Generated by N.Z. in Biorender. ATP, adenosine triphosphate; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; c-Fos, Protein c-Fos; ERK1/2, extracellular signal regulated protein kinase; FND5, fibronectin type III domain-containing protein 5; FoxO, Forkhead box protein O; Glu, glucose; KA, kynurenic acid; KATs, kynurenine aminotransferase; KYN, kynurenine; PGC-1α, proliferator-activated receptor gamma coactivator 1-alpha; PPARα/δ, peroxisome proliferator-activated receptor; QA, quinolinic acid; SIRT1, silent information regulator 1; TrkB, tyrosine kinase B receptor; TRP, tryptophan; VEGF, vascular endothelial growth factor.

range of biological effects, including neurogenesis, neural differentiation, and memory formation.<sup>8</sup> Irisin has the ability to cross the BBB and to exert its effect on brain tissue, as demonstrated after the stimulation of FND5 (peripheral delivery of FND5 to the liver via adenoviral vectors) in mouse liver and subsequent increases in circulating irisin and BDNF expression in the hippocampus.<sup>8</sup> As such, the PGC-1α/FND5/BDNF pathway represents a plausible avenue by which exercise promotes neurogenesis.<sup>23</sup> It has been proposed that the PGC-1α/FND5/BDNF pathway, and its positive effects on cognitive function, could be explained by its links to genes involved in cell growth, proliferation, and survival in animal brain.<sup>23</sup> These genes include PGC-1α, mammalian target of rapamycin (*mTOR*), extracellular signal regulated protein kinase (*ERK*), silent information regulator 1 (*Sirtuin 1*

or *SIRT1*), protein c-Fos (*c-Fos*), activity-regulated cytoskeletal-related gene (*ARC*), and forkhead box protein O (*FOXO*; Figure 3).<sup>23</sup>

## 4.2 | Exercise intensity and FND5

The effect of exercise on the FND5/irisin pathway has become the subject of growing research interest due to the beneficial effects on brain health. In a study by Pang et al.,<sup>24</sup> it was reported that 1 hour of treadmill running at a moderate intensity (15 m/minute, corresponding to 60% of  $\dot{V}O_{2max}$ ) produced increased FND5 mRNA levels in the gastrocnemius muscle of 6-week-old mice. This increase was observed during (30 minutes and 1 hour) and after (1, 2, 3, 6, 12, and

24 hours post-exercise) the exercise session, with the highest levels recorded 24 hours after the completion of exercise. Pang et al.<sup>24</sup> also observed a gradual increase in FNDC5 protein level, which also peaked at 24 minutes after exercise. This emphasizes the crucial importance of post-exercise sample timing for accurate assessment of the FNDC5 response. Further support for the capacity of moderate-intensity aerobic exercise to increase FNDC5 mRNA and protein levels in muscle, hippocampus, serum, and adipose tissue, in both human and rodent models, can be seen in Table S1.

With regard to the effects of exercise intensity, 8 weeks of moderate- to high-intensity training ( $4 \times 4$  minutes at an intensity of 80%–100% of the variation between the speed at lactate threshold and  $V_{\max}$ , 5 sessions per week) was superior ( $\approx 2$ -fold) to low-intensity training (28 minutes at 20% below speed at lactate threshold) in increasing FNDC5 protein level in rodent skeletal muscle.<sup>14</sup> Additionally, after a single high-intensity exercise session ( $5 \times 4$  minutes at  $\approx 80\%$  pre-training  $\dot{W}_{\max}$ ), FNDC5 mRNA has been reported to increase  $\approx 2$ -fold after 3 hours of recovery in moderately trained young men who were not currently engaged in a specific interval training protocol.<sup>25</sup> FNDC5 mRNA was increased  $\approx 3$ -fold in young male adults after 3 ( $8$  to  $22 \times 2$  to 4-minute intervals (power at lactate threshold [ $W_{LT}$ ] + 30%–80% of  $\dot{W}_{\max} - W_{LT}$ ) weeks of high-intensity training.<sup>25</sup> It is crucial to note that alterations in mRNA expression may not consistently align with changes in protein expression, underscoring the need for further investigation into the translation of these molecular changes.<sup>26</sup> In the single study comparing high-intensity and low-intensity exercise in humans across different age groups, no significant changes were observed in FNDC5 mRNA levels between the low- (cycling at 50%  $\dot{V}O_{2\max}$ ), high-intensity exercise (below the aerobic threshold for 15 minutes, between the aerobic–anaerobic thresholds [ $\approx$  second lactate threshold] for 10 minutes, above the anaerobic threshold for 5 minutes, and again below the aerobic threshold for 15 minutes), and control groups after one exercise session and after 21 weeks.<sup>27</sup> Notably, these “high-intensity” sessions primarily consisted of low-intensity exercise (30 minutes), with a minor portion of moderate (10 minutes) and high-intensity (5 minutes) exercise. In summary, one session of moderate-intensity treadmill running resulted in increased FNDC5 mRNA and protein levels in mice, with peak levels occurring 24 hours post-exercise, while longer voluntary and moderate-intensity exercise trainings consistently raised FNDC5 gene and protein levels across different tissues. Yet, it is unclear how high-intensity compares to moderate-intensity exercise training in both rodents and humans.

### 4.3 | Exercise intensity and irisin

Research investigating the effects of exercise on irisin levels across different tissues is limited, inconsistent, and warrants further investigation in both human and animal models. For example, a single session of low-intensity exercise (running at 60% of  $\dot{V}O_{2\max}$ ) increased, or had no effect, on serum/plasma irisin levels in rodents (Table S2). Furthermore, low-intensity exercise training (at  $\approx 65\%$ – $70\%$   $V_{\max}$  for 8 weeks) increased, or had no effect, on irisin concentrations in mice and rat

serum and skeletal muscle (Table S2). These inconsistencies are also apparent in humans, for which a single exercise session (running or pedaling at 45%–70% of  $\dot{V}O_{2\max}$ , categorized as “low” intensity based on Table 1) had no effect, increased, or decreased circulating irisin level (Table S2). Küster et al.<sup>28</sup> reported low-intensity exercise training ( $\approx 70\%$  of  $\dot{V}O_{2\max}$  for 12 weeks) had no effects on serum irisin level. In addition, Norheim et al.<sup>29</sup> reported an  $\approx 1.2$ -fold increase in circulating irisin levels after a single session of moderate-intensity exercise (45 minutes cycling at 75% of  $\dot{V}O_{2\max}$ ), but no change after 12 weeks of combined resistance and endurance training (no information provided regarding intensity). It is noteworthy that the intensity levels of these studies predominantly fall within the “low” to “moderate” range, rather than strictly “moderate,” and some studies did not specify the exercise intensity, such as Küster et al.<sup>28</sup> On the other hand, a single high-intensity exercise session reduced, or had no effect, on blood circulation irisin levels (Table S2). However, 12 weeks of high-intensity exercise training ( $> 90\%$   $\dot{V}O_{2\max}$ ) increased plasma concentrations of irisin levels in young healthy males (Table S2).

When comparing the effects of exercise intensity, moderate- and high-intensity exercise training (6–12 weeks) are equally effective for increasing irisin protein levels in rodent blood and muscle (Table S2). Conversely, in humans, a single session of high intensity is consistently reported to be superior, or at least as beneficial, at increasing blood irisin levels compared to a single session of moderate-intensity exercise (Table S2). However, Winn et al.<sup>30</sup> used exercise intensities of 55% and 80% of  $\dot{V}O_{2\max}$ , which better align with “low” and “moderate” intensity, respectively. Neither of these intensity levels would be considered “high intensity” based on our definition. Taken together, while the data remain inconclusive, there is emerging evidence suggesting that high-intensity exercise (especially when more precisely defined) may have a superior impact on irisin levels compared to lower intensities.

## 5 | CTSB

### 5.1 | The role of CTSB

CTSB is a lysosomal cysteine protease secreted during exercise that can cross the BBB and has been shown to have beneficial effects on BDNF expression in the brain.<sup>10</sup> Its primary roles are to promote clearance of neural debris and AHN (processes in which CTSB are involved in memory function).<sup>10</sup> In addition, CTSB is engaged in neuronal survival and has a significant anti-amyloidogenic activity.<sup>10</sup> The anti-amyloidogenic activity of CTSB suggests that it may help regulate the accumulation and deposition of amyloid proteins, such as amyloid beta, which are associated with neurodegenerative disorders.<sup>10</sup> By reducing the formation or promoting the clearance of amyloid aggregates, CTSB may contribute to the maintenance of neuronal health and the prevention of neurodegenerative pathologies.<sup>10</sup> In addition to its direct effect on BDNF, CTSB mediates processes related to cognition (e.g., synaptic plasticity, cell survival, and neuronal migration) through the induction of doublecortin (DCX), a protein involved in neurogenesis.<sup>10</sup> In addition, intravenous injection of recombinant



CTSB into CTSB knockout mice caused a significant increase in brain CTSB concentrations, demonstrating that CTSB can cross the BBB.<sup>10</sup> In support of the beneficial effects of CTSB on neurogenesis and cognitive function, exposing hippocampal progenitor cells to CTSB improved BDNF expression in the hippocampus of mice, and deletion of CTSB abolished the improvement of AHN and spatial memory function in response to exercise.<sup>10</sup> It has also been observed that CTSB transcripts are significantly upregulated in skeletal muscle cells upon the activation of both AMPK and PGC-1 $\alpha$ .<sup>31</sup> This may also indicate that exercise intensity might have a role in neuroprotective markers, considering that training intensity has been reported to modulate PGC-1 $\alpha$  protein content.<sup>15</sup>

## 5.2 | Exercise intensity and CTSB

Research regarding the impact of exercise intensity on CTSB levels is limited and inconclusive, highlighting the necessity for additional studies in both human and animal models. Moon et al.<sup>10</sup> investigated the effect of low- to moderate-intensity exercise training on CTSB levels in mice (voluntary wheel running, for 14 and 30 days) and monkeys (0.25-mile walks, 5 days/week, for 4 months). They reported that plasma CTSB levels significantly increased after 14 and 30 days in mice and after 16 weeks in monkeys. Additionally, gene expression and protein levels increased in mouse gastrocnemius muscle after 30 days of running and in monkey plasma after 4 months of training.<sup>10</sup> Conversely, 7 weeks of low- to moderate-intensity exercise (60–95 minutes running at 15 m/minutes, 5/week) had no effect on mice skeletal muscle CTSB protein levels.<sup>32</sup>

Despite the potential role of exercise intensity in regulating CTSB content (i.e., via PGC-1 $\alpha$ ), there is currently no research investigating the effects of moderate- or high-intensity exercise (or training) on CTSB levels in rodents. The one study investigating the effects of low- to moderate-intensity exercise on CTSB levels in humans reported that 4 months of training (45–75 minutes running at 70%–90% of HR<sub>max</sub>, 3/week) significantly increased plasma CTSB levels.<sup>10</sup> In addition, Mazo et al. reported an increase in serum CTSB level after one session of moderate- to high-intensity exercise (at 80% and 100% VO<sub>2max</sub>), but not low intensity (at 40% and 65% VO<sub>2max</sub>).<sup>33</sup> The former study also reported an increase in skeletal muscle CTSB protein levels 3 hours after exercise, and no change in CTSB mRNA level (Table S3).<sup>33</sup>

In summary, moderate-intensity exercise has been observed to increase CTSB protein levels in the plasma and skeletal muscles of humans, mice, and monkeys. Research investigating the effects of high-intensity exercise on CTSB levels is lacking, with only one study reporting increased plasma CTSB protein levels in humans after a single exercise session and no study investigating the effect of high-intensity training. Further research investigating the impact of exercise intensity on CTSB levels in diverse tissues, including muscle, brain, and blood, is warranted to fully understand its role in muscle–brain crosstalk, and further elucidate the role of exercise intensity on CTSB regulation.

## 6 | VEGF

### 6.1 | The role of VEGF

VEGF is a neurotrophic factor and signaling protein that is expressed in a wide range of cells and tissues, including smooth and skeletal muscle, endothelial cells, macrophages, astrocytes, and neuronal stem cells. There are two specific tyrosine kinase receptors for VEGF (VEGFR1 and VEGFR2),<sup>34</sup> which activate the same signaling cascades as BDNF. VEGF derived from both muscle and endothelial cells crosses the BBB and promotes angiogenesis and neurogenesis within the brain, particularly in the hippocampus. In neurons, VEGFR2 stimulation activates the PLC $\gamma$ /MAPK pathway. In astrocytes and microglia, VEGF activates MAPK/ERK via stimulation of VEGFR2, and PI3K by interaction with VEGFR1,<sup>34</sup> which in turn upregulates BDNF and ultimately improves cognitive function (Figure 3). VEGF stimulates the proliferation and survival of endothelial cells and acts as a nitric oxide-dependent vasodilator. Additionally, it plays a crucial role in promoting the formation of vascular networks and enhancing vascular permeability, which is important for maintaining optimal cerebral blood flow.<sup>34</sup>

During exercise, increased metabolic demand and muscle contraction itself prompt the release of various signaling molecules and factors, including VEGF. The mechanical strain on muscle fibers during exercise stimulates the production and release of VEGF.<sup>35</sup> VEGF plays a well-defined role in angiogenesis, as exercise-induced hypoxia prompts its release, leading to the formation of new blood vessels to support skeletal muscle adaptation and enhance the vascular network within the muscle tissue.<sup>35</sup> Similar to CTSB, it is hypothesized that activation of both AMPK and PGC-1 $\alpha$  results in a downstream increase in VEGF mRNA in skeletal muscle cells.<sup>31</sup> Lezi et al.<sup>36</sup> reported a strong positive correlation between VEGF and PGC-1 $\alpha$  mRNA levels. Considering exercise intensity has been reported as a regulator of PGC-1 $\alpha$  protein content,<sup>15</sup> and despite an increase in mRNA level not always corresponding to an increased protein level,<sup>26</sup> it is possible that high-intensity training may be superior to moderate-intensity training in activating VEGF.<sup>36</sup>

### 6.2 | Exercise intensity and VEGF

When investigating the effects of exercise on VEGF levels in rodents, low- to moderate-intensity exercise training (12–22 m/minutes treadmill running, 5/week for 8 weeks) has been reported to increase VEGF muscle mRNA levels in rats.<sup>37</sup> A single session of high-intensity exercise (24 m/minute, 10° incline) increased skeletal muscle and brain VEGF mRNA and protein levels in mice.<sup>38</sup> Furthermore, 7 weeks of moderate- to high-intensity training (42 minutes running at 18–25 m/minute, 2/days, 5/weeks) increased brain VEGF mRNA level in mice.<sup>36</sup>

Research investigating the effects of low- to moderate-intensity exercise training on VEGF protein levels in human blood is unclear, as studies have reported no change or increased serum and muscle VEGF

levels, respectively, after a single session of low-intensity exercise (30–60 minutes running at 60%–80% of  $HR_{max}$  or 62% of  $\dot{V}O_{2max}$ ; Table S4). Conversely, 12 weeks of low-intensity exercise training (30 minutes running at 65%–80%  $HR_{max}$ , 3/week) had no effects on circulating VEGF level.<sup>39</sup>

Research comparing low-to-moderate and high-intensity exercise training (1–8 weeks) on VEGF protein and mRNA levels in rodents is contradictory. For example, high-intensity exercise training has been reported to be superior to, as beneficial as, or inferior to, low-to-moderate-intensity training in VEGF levels in mice and rats (Table S4). Data in humans are also contradictory, as CTSB levels were increased in muscle (mRNA) and serum (protein) after both interval and continuous sprints,<sup>40</sup> but was also reported to be more elevated in skeletal muscle after a single session of moderate-intensity exercise compared to high-intensity exercise.<sup>41</sup>

In summary, the effects of exercise on VEGF levels in different rodent and human tissues are diverse, with a single session of high-intensity exercise generally increasing VEGF mRNA levels in rodents, but the clear role of exercise intensity is not entirely clear. These mixed results highlight the need for more research in this field, specifically research that investigates the role of exercise intensity (including low, moderate, and high intensity) in regulating VEGF levels in muscle, brain, and blood (Table S4).

## 7 | KYNURENINE METABOLITES

### 7.1 | The role of kynurenine metabolites

The kynurenine pathway plays a key role in muscle–brain crosstalk and is responsible for the degradation of  $\approx$  90% to 95% of TRP, an essential amino acid, in mammals. Subsequently, kynurenine is metabolized in one of the two main branches: (1) KA, which is considered a neuroprotective metabolite, and (2) 3-hydroxykynurenine (3HK), which ultimately will be metabolized to quinolinic acid (QA), a neurotoxic metabolite. Dysregulation of enzymes within the kynurenine pathway and perturbations in the concentration of its metabolites are implicated in the pathogenesis of several diseases, including Alzheimer's disease (AD) and Huntington's disease, schizophrenia and bipolar disorder, metabolic disorders, cardiovascular disease, and cancer.<sup>42</sup> Furthermore, alterations in these metabolites have been associated with aging and cognitive impairment.<sup>42</sup>

Concentrations of QA in the brain and cerebrospinal fluid are usually < 100 nm; however, in pathological conditions, such as Huntington's disease, AD, and acquired immune deficiency syndrome dementia complex,<sup>43</sup> concentrations of 500 to 1200 nm have been documented.<sup>43</sup> It has been reported that QA treatment within physiological concentrations (< 100 nm) elicits an increase in intracellular production of nicotinamide adenine dinucleotide ( $NAD^+$ ), an important cofactor in a variety of metabolic processes, in primary neurons and astrocytes of humans.<sup>44</sup> However, sustained elevation of QA levels can lead to neurotoxic effects through various mechanisms. In pathophysiological concentrations, QA activates N-methyl-D-aspartate (NMDA) receptors, which are a type of ionotropic receptors. This activation

increases the release of glutamate from neurons, inhibiting its uptake by astrocytes and suppressing the activity of astroglial glutamine synthetase. Consequently, there is an imbalance in microenvironment glutamate concentrations.<sup>45</sup> Prolonged activation of NMDA receptors and the resulting disproportionate microenvironment glutamate concentration can be detrimental to neurons, leading to excitotoxicity. Moreover, QA can induce lipid peroxidation,<sup>42</sup> cellular structural protein phosphorylation causing cytoskeleton destabilization, astroglial dysfunction, and gliotoxicity.<sup>42</sup>

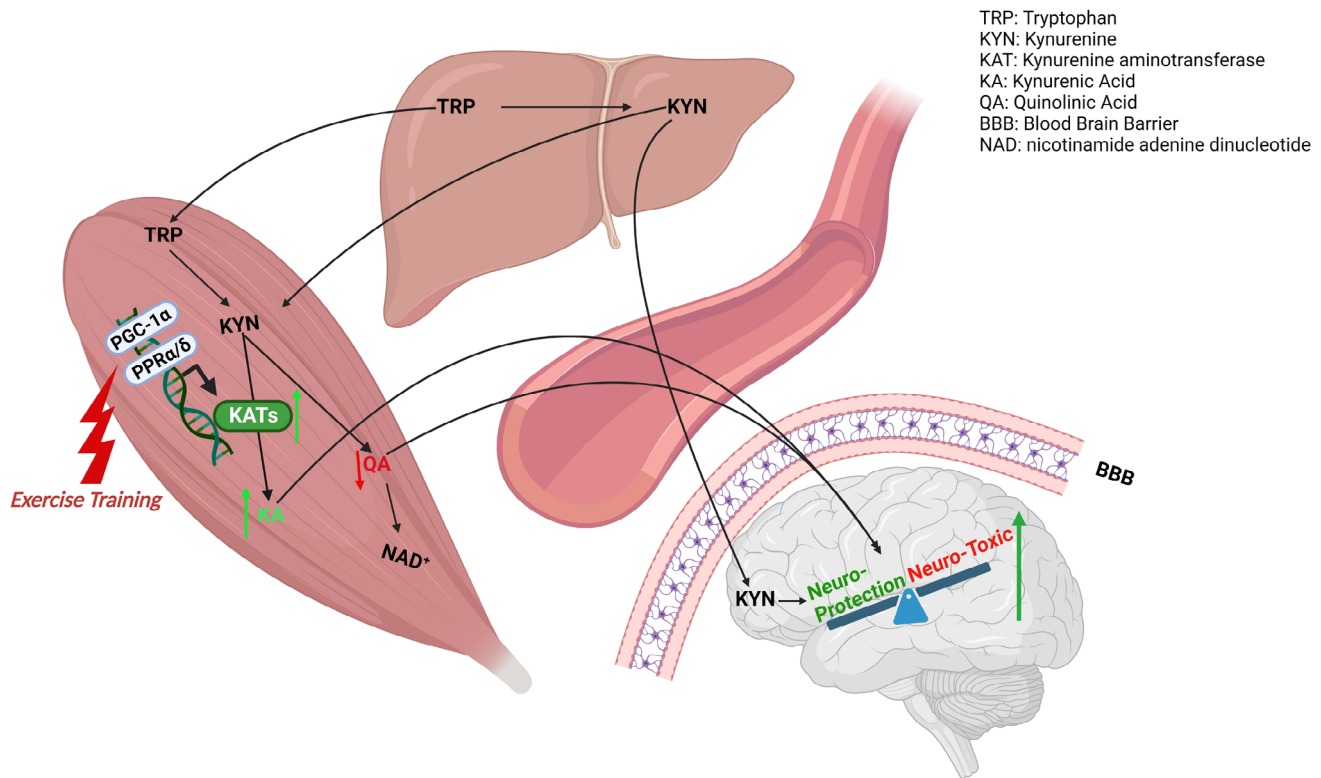
Conversely, KA, a NMDA receptor and  $\alpha 7$ nicotinic acetylcholine receptor antagonist, exerts its biological effects by reducing the extracellular release of glutamate.<sup>42</sup> In turn, this subsequently leads to a reduction in  $Ca^{2+}$  influx, and the prevention of neuronal damage. In short, the effects of the kynurenine pathway on neurotoxicity or neuroprotection are, at least in part, regulated by glutamatergic neurotransmission.

It has been reported that exercise-induced activation of the PGC-1 $\alpha$ –PPAR $\alpha/\delta$  (peroxisome proliferator-activated receptor) pathway stimulates the expression and content of KATs in skeletal muscle.<sup>46</sup> Considering KATs catalyze the conversion of kynurenine to KA, increased KAT content in skeletal muscle reduces plasma kynurenine. As a result, kynurenine availability, and the subsequent potential for QA (neurotoxic) accumulation within the brain is decreased (Figure 4).<sup>46</sup> In support of this, specific skeletal muscle PGC-1 $\alpha$  stimulation (generated by standard DNA microinjection) decreased plasma kynurenine and certain kynurenine metabolite concentrations in the hippocampus.<sup>46</sup>

### 7.2 | Exercise intensity and kynurenine metabolites

Considering the impact of training intensity on PGC-1 $\alpha$  protein content,<sup>15</sup> high-intensity training may be superior in promoting neuroprotective metabolites within the kynurenine pathway, highlighting the potential benefits of this exercise type for neuroprotection. Most of the research to date investigating the effects of exercise training on kynurenine metabolites is limited to low- to moderate-intensity training interventions,<sup>47</sup> as reported in both human and rodent models (Table S5). One session of low-intensity cycling (45 minutes at 60% peak power output or  $\dot{V}O_{2max}$ ) did not change kynurenine, TRP, KA, 3-HK, 3-hydroxyanthranilic acid (3-HAA), anthranilic acid (AA), picolinic acid (PA), and QA levels or increased KA and QA levels in humans (Table S5). For longer interventions, multiple studies have reported no effect of 3 weeks of low- to moderate-intensity exercise training on circulating kynurenine, TRP, and KA or increased circulating KA and skeletal muscle KAT 1-4 protein levels (Table S5).

High-intensity exercise training (3 weeks) increased KATIII, but not KATI and II protein, in human skeletal muscle, but had no effect on KA and QA in human blood.<sup>48</sup> Bansi et al.<sup>49</sup> and Joisten et al.<sup>50</sup> reported no changes in TRP, KYN, QA, or KA levels in human blood upon 3 weeks of low- versus high-intensity exercise. It is noteworthy that most of these studies measured kynurenine metabolites in the blood only, meaning the effects of exercise intensity on kynurenine metabolites in skeletal muscle, brain, and other tissues, are unclear.



**FIGURE 4** Exercise and the kynurenine pathway (interaction between peripheral tissues and the brain). Activation of the PGC-1 $\alpha$ -PPAR $\alpha/\delta$  pathway during exercise increases KATs in skeletal muscle. Enhanced KAT activity converts kynurenine to KA, reducing plasma kynurenine levels. This decreases KYN availability in the brain, potentially lowering QA accumulation. Generated by N.Z. in Biorender. KA, kynurenic acid; KATs, kynurenine aminotransferase; KYN, kynurenine; PGC-1 $\alpha$ , proliferator-activated receptor gamma coactivator 1-alpha; PPAR $\alpha/\delta$ , peroxisome proliferator-activated receptor; QA, quinolinic acid; TRP, tryptophan.

## 8 | AHN

### 8.1 | The role of AHN

There is emerging evidence to suggest a link between myokines (e.g., BDNF) and AHN,<sup>51</sup> and, as such, a secondary aim of this review was to investigate the role of exercise intensity on AHN. AHN is characterized by adult neural stem cells developing into functionally matured neurons through cell proliferation, differentiation, and functional maturity.<sup>52</sup> It plays a major role in memory development by promoting the connection of new neurons into the existing neural circuits, as well as maintaining the brain morphology and functional plasticity that deteriorates with aging and is characterized by reduced synaptic density and altered dendritic arborisation.<sup>52</sup>

To assess AHN, researchers typically use various histological and molecular techniques, among others.<sup>53</sup> These include:

- Cell proliferation: using markers, such as bromodeoxyuridine (BrdU+) or Ki67, to label actively dividing cells and quantify the rate of cell proliferation.
- Differentiation: The progression of neural stem cells into immature neurons (neuroblasts) can be evaluated by detecting markers like DCX.

- Neuronal survival: The survival of newly generated neurons can be determined by assessing the presence of mature neurons using markers like NeuN.
- Neurotrophic factors: The expression levels of neurotrophic factors, such as BDNF, can be measured to evaluate their role in supporting neurogenesis and neuronal survival.

AHN can be affected by a wide range of physiological and pathological factors, including exercise training and neuro-degenerative disorders.<sup>52</sup> These changes are regulated by a number of signaling pathways, including glutamatergic (primary excitatory neurotransmitters) and GABAergic (primary inhibitory neurotransmitters) inputs.<sup>52</sup> Exercise has been reported to enhance the expression of glutamate receptor subunits genes, such as *NR2A* and *NR2B*, which are highly expressed in newly generated neurons and promote long-term potentiation.<sup>54</sup> Conversely, it is thought that exercise modulates inhibitory neurotransmission (e.g., GABA receptor subunits), which may attenuate anxiety and improve cognition.<sup>54</sup> Being exposed to an enriched environment activates immature neurons through GABAergic inputs, and subsequent activation of NMDA receptors, which allows immature neurons to respond to future glutamatergic synaptic inputs.<sup>52</sup>

For the sake of this article, we focus on AHN in animal models, evaluated through established markers discussed in the previous sections.

However, it is noteworthy that there have been attempts in human randomized controlled trials to measure neurogenesis via advanced imaging techniques such as magnetic resonance imaging (MRI). While MRI cannot directly quantify neurogenesis, surrogate measures, such as hippocampal volume and structural connectivity, have been used as indirect indicators of neurogenesis. For example, Erickson et al.<sup>55</sup> demonstrated that aerobic exercise can increase hippocampal volume in older adults, suggesting a potential link to enhanced neurogenic processes. However, these approaches remain limited by the indirect nature of the measurements and the inability to capture cell-level processes like proliferation or differentiation.

More recently there has been growing interest in how exercise training promotes AHN. Exercise training improves blood flow and delivery of oxygen and nutrients to the brain stimulating neurogenic effects.<sup>11</sup> It also enhances cell proliferation by shortening the cell cycle length, amplifying progenitor cells, accelerating functional maturation, and preventing the death of new neurons.<sup>54</sup> In addition, spine formation, dendritic spine motility, total dendritic length, branch points, and dendritic complexity are all enhanced after exercise training.<sup>54</sup> Myokines, secreted by skeletal muscle during exercise, have been implicated in promoting neuroplasticity and cognitive function, potentially influencing AHN dynamics. As such, another aim of this paper was to investigate the role of exercise in promoting AHN, with particular emphasis on exploring the effects of exercise intensity. It is worth noting that previous research investigating exercise and AHN is solely from intervention studies in rodents (due to accessibility to brain tissue), which provides obvious limitations in the translation of findings to humans.

## 8.2 | Exercise intensity and AHN

There is evidence that free wheel running (which we have defined as low- to moderate-intensity) training (1–12 weeks) significantly increases AHN (Table S6). Fabel et al.<sup>56</sup> reported that 1 week of free wheel running increased the total number of BrdU<sup>+</sup> cells within the dentate gyrus of mice. In addition, it was reported that 4 weeks of free wheel running can increase the number of BrdU<sup>+</sup> cells in both 3- and 11-month-old mice.<sup>31</sup> This study also reported that the number of newly generated mature neurons (determined by NeuN<sup>+</sup>/BrdU<sup>+</sup> co-labelling) improved in 3- and not in 11-month-old mice.<sup>31</sup> In the only study investigating the effects of high-intensity exercise training alone (7 weeks), AHN was unchanged.<sup>57</sup> Nokia et al.<sup>57</sup> also showed that high-intensity training (20 minutes running, 3 minutes at 85%–90% of  $V_{max}$  with 2 minutes recovery at 50% of max speed, 3 p/week, for 7 weeks) did not change DCX<sup>+</sup> cells. In summary, strong evidence supports that low- to moderate-intensity exercise training is a strong stimulus to enhance AHN.

There are only four studies that have directly compared the effects of different exercise training intensities on AHN. Collectively, moderate-intensity exercise training appears to improve AHN to a greater extent than high-intensity training; however, more evidence is needed (Table S6). For example, 1 week of treadmill exercise at

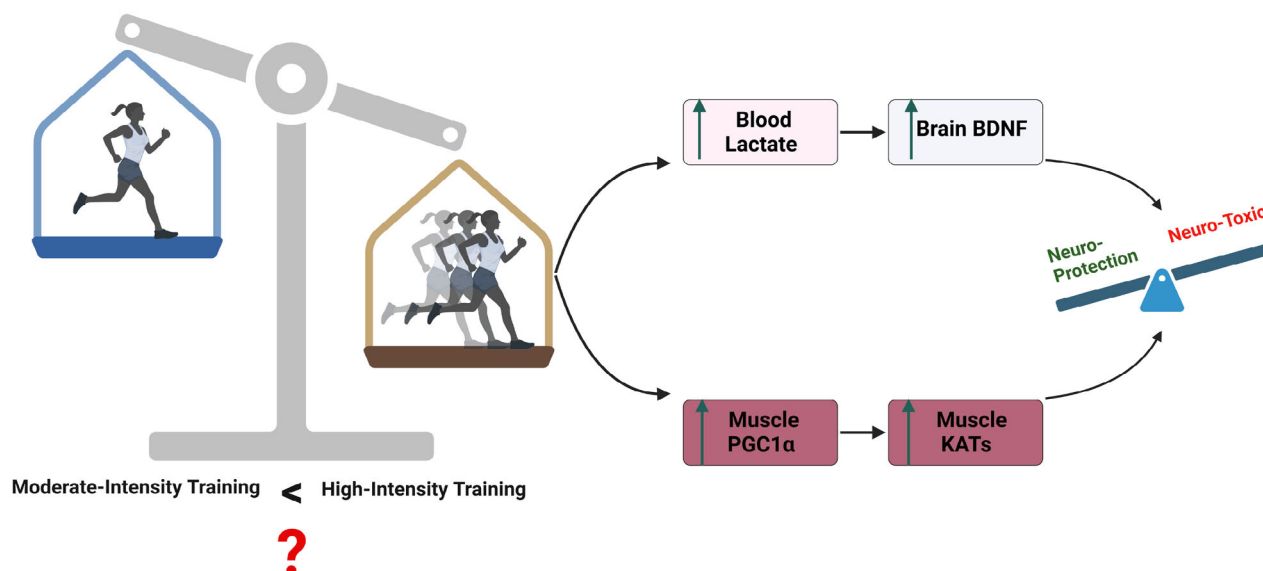
what was classified as moderate intensity (30 minutes running at 14 m/minute) or low intensity (30 minutes running at 11 m/minute) led to enhanced neurogenesis in the dentate gyrus of the hippocampus of rats. However, no changes were observed in what was classified as high-intensity exercise (30 minutes at 10–22 m/minute).<sup>58</sup> In support of this, BDNF mRNA was increased in the low-, but not high-intensity training group after 1 week of an exercise intervention.<sup>58</sup> In another study, 6 weeks of low-intensity exercise training (15 m/minute), but not high-intensity exercise (40 m/minute), improved AHN, specifically the promotion of neuronal maturation of newly born cells in 11-week-old male Wistar rats.<sup>59</sup>

In the study by So et al.,<sup>60</sup> 6 weeks of moderate-intensity exercise training (40 minutes/day at 15 m/minute) enhanced cell proliferation, survival, neuronal differentiation, and migration, whereas high-intensity exercise training (40 minutes/day—speed was increased by 5 m/minute every 1 minute, until it reached 30 m/minute) promoted neuronal differentiation and migration only, in 8-week-old female mice. This phenomenon was accompanied with a reduction in mitochondrial activity (using prohibitin as a marker of mitochondrial activity in the dentate gyrus), and lower concentrations of VEGF, BDNF, and insulin-like growth factor 1 protein levels, in the high-intensity group compared to the moderate-intensity group.<sup>60</sup> These inconsistencies may be explained, at least in part, by differences in the training mode (e.g., treadmill, running wheel, swimming, and so forth) and/or other exercise prescription variables (e.g., duration and total work performed). Moreover, it is possible that high-intensity exercise exerts differential effects on AHN due to its unique physiological demands. High-intensity exercise may induce transient increases in systemic and central inflammation, mediated by pro-inflammatory cytokines, which could counteract some of the beneficial effects typically associated with exercise. Additionally, the elevated release of stress hormones, such as cortisol, during high-intensity exercise could negatively impact hippocampal plasticity, as excessive activation of the hypothalamic–pituitary–adrenal axis is known to inhibit neurogenesis.<sup>60</sup> In summary, low- to moderate-intensity exercise training interventions appear to promote an increase in AHN, whereas the effects of high-intensity exercise training on AHN are less studied.

## 9 | SUMMARY AND CONCLUSIONS

It is well established that exercise has beneficial effects on brain health (Figure 1).<sup>6</sup> However, the most effective intensity of exercise to promote positive effects on brain health, specifically the underlying molecular mechanisms involved in muscle–brain interactions, are yet to be fully elucidated. The aim of this review paper was to highlight the potential role of exercise intensity on muscle-derived mediators of neuroprotection, with an additional focus on their role in regulating BDNF and AHN (Figure 5).

The current body of evidence is insufficient to draw definitive conclusions regarding the impact of exercise intensity, particularly high-intensity training, on exercise-induced neuroprotective media-



**FIGURE 5** Exercise intensity and neuroprotective myokines. High-intensity exercise may lead to great increases in blood lactate, promoting lactate-dependent upregulation of BDNF via myokines. Furthermore, high-intensity exercise enhances PGC-1 $\alpha$ -induced KAT content in muscle, reducing neurotoxicity and promoting neuroprotective myokine pathways. Generated by N.Z. in Biorender. BDNF, brain-derived neurotrophic factor; KAT, kynurenine aminotransferase; PGC-1 $\alpha$ , proliferator-activated receptor gamma coactivator 1- $\alpha$ .

tors derived from muscle and kynurenine pathway metabolites. Future research, with adequate controls in place (e.g., work-matched training interventions), is necessary and warranted.

In addition, the importance of accurately defining exercise intensity in research protocols to specifically elicit the desired metabolic stimulus and facilitate clearer interpretation of study outcomes and comparisons across studies is crucial. For instance, to study the effects of exercise intensity on FNDC5 protein levels in rodents, Constans et al.<sup>14</sup> compared 8 weeks of “high-intensity” training (4  $\times$  4 minutes at an intensity of 80%–100% of the variation between the speed at lactate threshold and  $V_{\max}$ , which we would describe as “moderate to high”), with a group that performed “moderate-intensity” training (28 minutes at 20% below the speed at the first lactate threshold, which we would consider “low” intensity). Similar inconsistencies are apparent in studies that have attempted to assess the effect of exercise intensity on VEGF levels in different tissues. For example, Lou et al.<sup>58</sup> categorized exercise at 14 m/minute as “moderate” (which, according to Table 1, would align more closely with “low” intensity), and Wang et al.<sup>61</sup> classified exercise at 40% of  $V_{\max}$  as “moderate” although it predominantly falls within the “low” intensity range. These are just a few examples that underscore the importance of accurately identifying and defining exercise intensity levels, as misclassification can lead to misinterpretation of study outcomes and hinder comparisons across studies.

Understanding the influence of exercise intensity on the regulation of myokines implicated in exercise-induced neuroprotection represents a critical avenue for further exploration. Improving our understanding of the role of myokines in regulating brain health holds significant promise in offering therapeutic avenues to alleviate the burden of neurodegenerative conditions (e.g., AD).

This review focused on aerobic exercise, as the majority of literature investigates its effects on brain health. However, emerging studies suggest resistance training may also positively impact cognition, potentially through mechanisms like myokine secretion and reduced inflammation. Although data on resistance training intensity are limited, future research should explore its role in neuroprotection and muscle–brain crosstalk, particularly compared to aerobic exercise. In addition, future research should aim to integrate findings from clinical trials and longitudinal studies examining the effects of different exercise intensities, including HIIT and moderate continuous training, on brain-related functional outcomes in populations with AD, dementia, and other neurological conditions such as Parkinson’s disease. Such investigations could provide critical insights into whether exercise-induced myokine responses observed in healthy populations translate to improved neuroplasticity, synaptic strength, or brain blood flow in clinical contexts. This could also help elucidate the differential benefits of exercise dosing in disease-associated brain health outcomes and further solidify the clinical relevance of myokines in these populations. Additionally, this review does not cover all myokines implicated in exercise and neuroplasticity, leaving an opportunity for future comprehensive reviews to explore these pathways.

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## CONFLICT OF INTEREST STATEMENT

All authors declare that they have no conflicts of interest to disclose. Author disclosures are available in the [supporting information](#).



## CONSENT FOR PUBLICATION

Appropriate author consents have been obtained.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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