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REVIEW

Exercise and circulating BDNF: Mechanisms of release and implications for the design of exercise interventions[1]

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Abstract: Engagement in regular bouts of exercise confers numerous positive effects on brain health across the lifespan. Acute bouts of exercise transiently improve cognitive function, while long-term exercise training stimulates brain plasticity, improves brain function, and helps to stave off neurological disease. The action of brain-derived neurotrophic factor (BDNF) is a candidate mechanism underlying these exercise-induced benefits and is the subject of considerable attention in the exercise–brain health literature. It is well established that acute exercise increases circulating levels of BDNF and numerous studies have sought to characterize this response for the purpose of improving brain health. Despite the interest in BDNF responses to exercise, little focus has been given to understanding the sources and mechanisms that underlie this response for the purpose of deliberately increasing circulating levels of BDNF. Here we review evidence to support that exploiting these mechanisms of BDNF release can help to optimize brain plasticity outcomes via exercise interventions, which could be especially relevant in the context of multimodal training (i.e., exercise and cognitive stimulation). Therefore, the purpose of this paper is to review the candidate sources of BDNF during exercise and the mechanisms of release. As well, we discuss strategies for maximizing BDNF responses to exercise, and propose novel research directions for advancing our understanding of these mechanisms.

Key words: brain plasticity, platelets, physical activity, acute exercise, vascular endothelial cells.

Résumé : La participation régulière à des séances d'activité physique procure durant toute la vie de nombreux bienfaits pour la santé du cerveau. Des séances ponctuelles d'activité physique améliorent provisoirement la fonction cognitive et l'entraînement physique à long terme stimule la plasticité du cerveau, améliore la fonction cérébrale et contribue à repousser les maladies neurologiques. L'action du facteur neutrophique dérivé du cerveau (« BDNF ») serait associée au mécanisme sous-jacent du bienfait suscité par l'exercice physique et suscite un intérêt marqué dans la documentation sur la santé cérébrale et l'activité physique. Études à l'appui, un exercice ponctuel accroit le taux sanguin de BDNF et de nombreuses études cherchent à préciser cette action afin d'améliorer la santé cérébrale. Nonobstant cet intérêt à l'égard de la réponse du BDNF à l'exercice physique, il y a peu d'études traitant des sources et des mécanismes sous-jacents dont l'objectif est d'accroître le taux sanguin de BDNF. Dans cet article, on présente les données probantes selon lesquelles on peut stimuler les mécanismes de libération du BDNF pour optimiser les résultats de la plasticité cérébrale au moyen d'interventions kinésiologiques lesquelles sont particulièrement pertinentes dans le contexte de l'entraînement multimodal (c.-à-d. exercice physique et stimulation cognitive). En conséquence, cet article a pour objectif d'examiner les sources du BDNF au cours d'un exercice physique et les mécanismes de libération. De plus, nous traitons des stratégies pour maximiser les réponses du BDNF à l'exercice physique et nous proposons des orientations scientifiques novatrices pour approfondir les connaissances au sujet de ces mécanismes. [Traduit par la Rédaction]

Mots-clés : plasticité cérébrale, plaquettes, activité physique, exercice ponctuel, cellules endothéliales vasculaires.

Introduction

It is now well-established that both acute and chronic exercise positively impact brain structure and function across the lifespan (Smith et al. 2010; Voss et al. 2011*b*; Chang et al. 2012; Erickson et al. 2014; Verburgh et al. 2014). Higher aerobic fitness is associated with larger prefrontal cortex and hippocampal volumes in older adults (Erickson et al. 2014), and more efficient neural processing (Voss et al. 2011*a*) and greater white matter integrity in children (Chaddock-Heyman et al. 2014). Importantly, the brain retains the capacity to positively adapt to periods of exercise training in previously inactive young and older adults. These structural and physiological adaptations manifest as improvements in cognitive function (Pereira et al. 2007; Erickson et al. 2011). This is especially important for executive functions that govern flexible, decision-making behaviours in an ever-changing environment (Smith et al. 2010; Chang et al. 2012; Verburgh et al. 2014).

Advancements in understanding the specific mechanisms that underlie improved cognitive performance and neuroplasticity have also been made, and have been discussed in a number of excellent reviews (Cotman et al. 2007; Voss et al. 2011*b*; Hamilton and Rhodes 2015). Broadly speaking, exercise stimulates the release of neurotransmitters and neurotrophins in an activity-dependent manner, which acutely potentiates neural function and induces a cascade of events that promote structural and functional plasticity of the brain (Cotman et al. 2007; Wrann et al. 2013; Hamilton and Rhodes 2015). While a concert of mechanisms contribute to brain plasticity, the actions of brain-derived neurotrophic factor (BDNF) presents as one of the key mechanisms underlying exercise-induced brain plasticity and cognitive en-

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hancement (Berchtold et al. 2005; Cotman et al. 2007; Intlekofer et al. 2013).

BDNF is a protein that belongs to the neurotrophin family and is abundantly expressed in the hippocampus and cerebral cortex (Rasmussen et al. 2009; Quirié et al. 2012; Park and Poo 2013). BDNF is essential for brain development, for the proliferation and maintenance of neurons, and for cognitive functions such as learning and memory (Cirulli et al. 2004; Park and Poo 2013; Zagrebelsky and Korte 2014). A collective body of work in animals has shown that BDNF is obligatory for exercise-induced brain plasticity and cognitive function, such that blocking the tropomyosin receptor kinase B (TrkB) for BDNF over a 3-week period of exercise training abrogates these beneficial effects (Intlekofer et al. 2013). Interestingly, nonexercise upregulation of BDNF via sodium butyrate in sedentary rats mimics the positive effects of exercise on hippocampal function, supporting the role of BDNF in exercise-induced enhancement of learning and memory (Intlekofer et al. 2013).

Given the demonstrated importance of BDNF in animals, considerable effort has been made to determine whether exercise elevates circulating BDNF in humans. The best available evidence shows that acute aerobic exercise transiently increases circulating BDNF, while exercise training seems to have small and highly variable effects on resting concentrations (Knaepen et al. 2010; Szuhany et al. 2015; Dinoff et al. 2017). However, given that BDNF must access neural tissue to exert its effects (i.e., cross the blood-brain barrier (BBB)) and that in vivo measurement of BDNF in the human brain is impossible, studies in humans rely solely on the measure of circulating levels to make inferences regarding neural tissue bioavailability. BDNF is believed to cross the BBB in a bi-directional manner (Pan et al. 1998) and indirect evidence from animals and humans suggest that circulating BDNF is important for central function (Ziegenhorn et al. 2007; Erickson et al. 2010, 2012; Schmidt and Duman 2010; Angelucci et al. 2011; Polyakova et al. 2015).

Given the beneficial effects of exercise on the brain and that BDNF is a prime candidate molecule for mediating these effects, it would seem imperative to understand how exercise elevates BDNF to inform the development of the most effective modalities of exercise for maximizing circulating BDNF. However, little attention has been paid to understanding and connecting the mechanisms by which exercise might elevate BDNF to a given exercise modality. This is a critical shortcoming of the current body of literature, in that it prevents mechanism-based development of effective exercise modalities for maximizing BDNF. Understanding these mechanisms may have functional implications for an array of populations, and can strengthen the rationale and design of exercise interventions focused on improving brain health. Therefore, the purpose of this review is to (*i*) identify potential contributors to circulating BDNF with exercise and the mechanisms responsible for these contributions, and (*ii*) to apply this knowledge to the design of exercise interventions that are effective at elevating circulating BDNF. This review focuses specifically on BDNF responses to *exercise*, which is planned, structured, and repetitive body movement that increases energy expenditure with the objective of improving or maintaining fitness, as opposed to *physical activity*, which refers to any bodily movement that increases energy expenditure (>1.5 metabolic equivalents) above resting levels (Caspersen et al. 1985).

Exercise and BDNF

Acute exercise is a potent stimulus for increasing blood-borne BDNF in young, middle-aged, and older adults (Knaepen et al. 2010; Szuhany et al. 2015; Dinoff et al. 2017) and relative to pharmacological approaches, is cost-effective, safe, and easily accessible to the population at-large. In a recent meta-analysis, Szuhany et al. (2015) determined that a single bout of exercise (predominantly aerobic) has a moderate effect on plasma and serum BDNF (Hedges' g = 0.46), and that this acute effect is potentiated by a preceding period of regular exercise training (Hedges' g = 0.59). Similarly, Dinoff et al. (2017) reported an effect size of 0.59 in favour of acute exercise increasing BDNF, and that this response is augmented in those with higher cardiorespiratory fitness (peak oxygen uptake ($\dot{V}O_{2peak}$)). The augmented acute BDNF response in those with higher fitness may buffer the metabolic stress associated with increased energy turnover (Matthews et al. 2009; Walsh et al. 2015) and/or represent a beneficial evolutionary adaptation that supports successful hunting and foraging behaviours (Raichlen and Alexander 2017).

The bulk of available evidence has primarily focused on acute aerobic exercise paradigms; however, it appears as though both acute aerobic and resistance exercise modalities are effective at increasing circulating BDNF (Dinoff et al. 2017). The aspects of exercise that drive the BDNF response are equivocal, as 1 systematic review suggests BDNF increases in an intensity-dependent manner (Knaepen et al. 2010), whereas others report that exercise duration drives this response (Dinoff et al. 2017). Regardless, this effect is relatively short-lived following cessation from exercise as levels return to baseline levels within 30 min for serum (Yarrow et al. 2010; Walsh et al. 2016) and 60 min for plasma (Gilder et al. 2014). Moreover, chronic exercise training has equivocal and highly variable effects on resting BDNF (Knaepen et al. 2010; Szuhany et al. 2015; Dinoff et al. 2016). Dinoff et al. (2016) concluded that a period of aerobic, but not resistance training, increases resting serum and plasma BDNF; however, only half of the reported studies (9/18) observed an increase in resting BDNF – an effect that was largely driven by studies that included populations that are known to have lower basal BDNF (i.e., Parkinson's disease, obesity, and metabolic syndrome).

Part 1 – Sources and mechanisms of BDNF release

Defining pools circulating of BDNF

Circulating BDNF exists in 2 distinct pools: (*i*) BDNF that is bound to platelets, and (*ii*) BDNF that circulates freely in plasma (unbound) (Fig. 1). Blood serum measures represent the total measurable blood-borne BDNF (bound and unbound) while plasma measures represent only the free (unbound) portion. The contribution of plasma BDNF to total circulating levels is considerably lower than serum, as serum contains ~100–200 times more BDNF than plasma (Rosenfeld et al. 1995); however, this small fraction of unbound BDNF represents the bioavailable pool that is free to associate with TrkB or p75 receptors (Fujimura et al. 2002; Zagrebelsky and Korte 2014).

Within the acute exercise and BDNF literature, serum is the most commonly measured blood parameter, followed by plasma, and platelets, respectively (Knaepen et al. 2010; Dinoff et al. 2017). In fact, of the 55 studies included in a recent meta-analysis, 42 measured serum alone, 9 measured plasma alone, and 4 measured both serum and plasma (Dinoff et al. 2017). The portion of blood (serum or plasma) that is selected for analysis has implications for the interpretation of data (Pareja-Galeano et al. 2015) since BDNF can move between bound and unbound pools without addition to or removal from the total circulating BDNF pool (Fujimura et al. 2002) (Fig. 1). This is especially relevant in the context of acute exercise, as conditions in the local physiological milieu of an active tissue bed (i.e., skeletal muscle or brain) can influence the offloading and uptake of BDNF by platelets (Fujimura et al. 2002). The best way to fully characterize the BDNF response to exercise would include measurements of serum, plasma, platelets, and a calculation of the amount of BDNF per platelet. This would allow for evaluation of the unbound portion relative to changes in total BDNF and the calculation of the amount of BDNF per platelet, which may provide evidence of de novo BDNF from a cellular source (Fig. 1).

Fig. 1. Sources of BDNF addition and removal from the circulating pool (from left to right): BDNF can be added to the circulating pool via tissue sources and through the addition of platelets to the blood via thrombocytosis (splenic constriction and platelet release). Circulating BDNF can move between the platelet-bound and the plasma pools in response to specific physiological stimuli. Plasma (free) BDNF can be removed from the blood by acting on the TrkB receptor and platelet-bound BDNF can be removed from the blood via the reuptake of platelets by the spleen. BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin-related kinase B. [Colour online.]

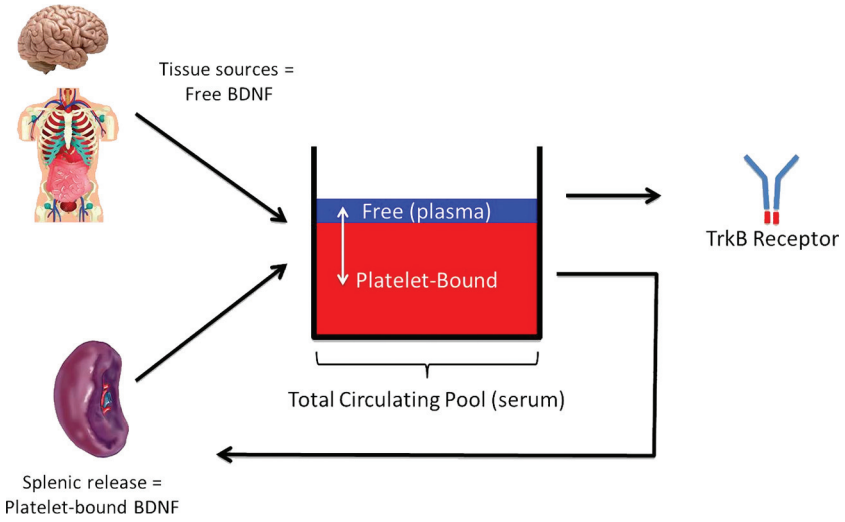


Table 1. Mechanisms of BDNF release in response to acute exercise.

Mechanism	Source of BDNF	Appearance time frame	Fate of BDNF
Thrombocytosis via SNA	Platelets[a,b] and PBMC via the spleen[b,c]	Fast (<5 min)[b,d,e]	Increased serum and plasma (by extension of release from platelets and PBMCs)
Shear stress via muscle and cerebral blood flow	Endothelial cells[f,g] and platelets[h]	Fast (5–6 min)[f,h]	Endothelial cells: increased serum via plasma Platelets: increased plasma
Hypoxia	Endothelial cells[i]	Fast (<10 min)[i]	Increased serum via plasma
Brain activity during exercise	Neurons[j,k,l] and endothelial cells[g,m]	Rapid (with neural firing)[j]	Increased serum via plasma
Energetic stress	Skeletal muscle[n,o] and neurons[p]	Delayed (3–48 h)[n,o,p]	Increased mRNA and protein content No change to blood
Hyperthermia	Δ BBB permeability[q] and endothelial cells[f,g]	Fast (within an exercise bout or 20 min of heating[r,s,t])	Increased serum via plasma

Note: List of mechanisms that contribute to circulating BDNF with exercise. Most sources contribute free, de novo BDNF to the plasma; however, the largest contribution of BDNF with exercise comes from platelets released via thrombocytosis. The time frame of release is based off the best available evidence, which reflects the native experimental design rather than a specific release time frame for BDNF. BBB, blood-brain barrier; BDNF, brain-derived neurotrophic factor; mRNA, messenger RNA; PBMC, peripheral blood mononuclear cells; SNA, sympathetic nerve activity. References: [a]Hulmi et al. 2010; [b]Chamberlain et al. 1990; [c]Brunelli et al. 2012; [d]Walsh et al. 2017; [e]Frances et al. 2008; [f]Prigent-Tessier et al. 2013; [g]Monnier et al. 2017; [h]Fujimura et al. 2002; [i]Helan et al. 2014; [j]Tanaka et al. 2008; [k]Bechara et al. 2014; [l]Seifert et al. 2010; [m]Guo et al. 2008; [n]Matthews et al. 2009; [o]Walsh et al. 2015; [p]Rasmussen et al. 2009; [q]Watson et al. 2005; [r]Kojima et al. 2017; [s]Goekint et al. 2011; [t]Lee et al. 2014.

Sources and mechanisms of BDNF release during exercise

The addition of BDNF to the blood at rest and during exercise is likely derived from a number of tissue sources that produce and release the neurotrophin into circulation in response to exercise-like stimuli. BDNF was originally purified in homogenized pig brain where it was recognized for its role in the growth and survival of sensory neurons (Barde et al. 1982); however, referring to this neurotrophin as "brain-derived" is somewhat misleading as it has been established that a host of tissues produce and release BDNF, in addition to the brain. These include the lungs, bladder, intestinal tissue, vascular endothelial cells, skeletal and cardiac muscle, peripheral neurons, peripheral blood mononuclear cells (PBMCs), and platelets (Lommatzsch et al. 1999; Fujimura et al. 2002; Matthews et al. 2009; Brunelli et al. 2012; Quirié et al. 2012; Prigent-Tessier et al. 2013; Marosi and Mattson 2014, Walsh et al. 2015). The role of BDNF in these tissues appears to be related to neural growth and survival (Lommatzsch et al. 1999), consistent with it being a neurotrophic factor, but also to modulation of smooth muscle tone (Prigent-Tessier et al. 2013), tissue remodelling (Kerschensteiner et al. 1999), and energy regulation (Matthews et al. 2009; Walsh et al. 2015). An important similarity between these tissue sources is that BDNF is released under conditions of physiological stress evoked by exercise (Table 1). In the context of exercise, the candidate tissues for the addition of BDNF to circulation are the brain, skeletal muscle, PBMCs, vascular endothelial cells, and platelets via the spleen.

Sources of plasma BDNF

The brain

Neurons produce and release BDNF in an activity-dependent manner (Pan et al. 1998; Tanaka et al. 2008; Marosi and Mattson 2014). The production and release is regulated by excitatory synaptic activity as well as the presence of specific hormones and neuropeptides (Marosi and Mattson 2014). Direct and indirect evidence supports the ability of BDNF to bi-directionally cross the BBB via a high-capacity saturable transport system (Poduslo and Curran 1996; Pan et al. 1998; Krabbe et al. 2007; Rasmussen et al. 2009; Schmidt and Duman 2010). However, it must be acknowledged that others have argued against this, stating that a lack of BDNF transporter on the cerebrovascular endothelium and the cationic nature of the protein render circulating BDNF unable to

cross the BBB and susceptible to rapid removal by the liver (Sakane and Pardridge 1997; Pardridge et al. 1998).

The commonly accepted viewpoint at present is that the brain is the primary source of circulating BDNF at rest and during exercise. This is based on a series of studies that measured arterial and jugular vein plasma BDNF concentration ([BDNF]) and quantified this venous-arterial difference as representing the amount of BDNF in the "venous circulation" that had originated from brain tissue release (Krabbe et al. 2007; Rasmussen et al. 2009; Seifert et al. 2010). Specifically, Krabbe et al. (2007) observed a jugular-arterial difference in [BDNF] of approximately 500 pg/mL at rest, which remained relatively stable over a 1-h period. Seifert et al. (2010) found that 30 min of acute cycling exercise at 70% maximal oxygen uptake ($\dot{V}O_{2max}$) increases the concentration of both arterial (systemic) and jugular venous plasma [BDNF]. The jugular-arterial difference in [BDNF] increased from 1300 pg/mL at rest to 2400 pg/mL during exercise in a group designated for eventual exercise training. Rasmussen et al. (2009) quantified the increase in plasma [BDNF] from arterial to jugular venous circulation as a proportion of jugular venous plasma [BDNF]. At rest the jugular venous plasma [BDNF] was more than 5-fold higher than arterial plasma [BDNF] (442 pg/mL vs. 95 pg/mL), indicating that a considerable amount of BDNF had been added to the plasma as it passed through the cerebral circulation. This addition accounted for 72% of jugular venous plasma [BDNF]. At 4 h of rowing ergometer exercise at 10%–15% below lactate threshold, the jugular venous plasma [BDNF] was ~2.5-fold greater than it had been at rest and addition of BDNF to plasma in the cerebral circulation accounted for 84% of jugular venous plasma [BDNF]. Again, jugular venous plasma [BDNF] was more than 4-fold greater than arterial (1172 pg/mL vs. 270 pg/mL). This led the authors to conclude that "almost three quarters of the BDNF present in the venous circulation originated from brain structures [and] suggests that brain tissue is the major contributor to circulating BDNF."

These highly cited findings are generally accepted as confirmation that the brain is the primary source of circulating BDNF at rest and during exercise. However, this interpretation needs to be viewed with caution. First, it must be recognized that the authors measured jugular venous plasma [BDNF] not venous blood leaving other tissue beds, nor the accumulation of all tissue venous effluent entering the right atrium of the heart. Thus, while the total amount of BDNF added to plasma in the cerebral venous effluent supports the contention that the brain releases BDNF, this release cannot be quantified as a proportion of the total circulating pool of BDNF. Second, platelet activation in the cerebrovascular bed could release BDNF. The authors do acknowledge this possibility. Given that platelets hold ~100–200 times more BDNF than plasma it would not require much activation to substantially increase plasma [BDNF].

Both of these considerations may account for why there is such a substantial reduction in plasma [BDNF] from the cerebral venous effluent to the arterial circulation. As the authors correctly point out, this could simply be dilution of the BDNF from the cerebral circulation when it mixes with all other tissue venous return. Or it could be because circulating platelets rapidly release but also sequester BDNF under different physiological stimuli, thereby having the potential to influence the plasma pool across the venous-arterial transit without addition or removal by tissue sources. The lone measurement of plasma could falsely indicate an increase in BDNF solely from a cellular source and not account for the partial contribution from platelets. Thus, while considerable increases in cerebral venous effluent plasma [BDNF] support the brain as a source of BDNF, the quantitative contribution to circulating BDNF may very well be much less than "major". Accordingly, the relative contribution of BDNF from the brain during exercise needs be re-examined using a more complete characterization of the BDNF system and more rigorous accounting for the potential peripheral tissue sources. The inclusion of serum, plasma, platelets, and a calculation of BDNF per platelet allows for this and account for possible movement of BDNF between pools without the addition of de novo BDNF.

Skeletal muscle

Skeletal muscle produces BDNF under conditions of energetic stress such as prolonged exercise (Matthews et al. 2009) and fasting (Walsh et al. 2015), where it is believe to be involved in the regulation of fat metabolism (Matthews et al. 2009). While an attractive candidate, there is currently evidence to suggest that skeletal muscle is not a secretory organ of BDNF. Through a series of animal and cellular experiments, Matthews et al. (2009) observed that while skeletal muscle contraction increases the expression of intramuscular BDNF, it acts in an autocrine/paracrine fashion. Mechanistically, this does not preclude the ability of BDNF to enter the circulation as intramuscular BDNF would have to signal TrkB extracellularly to exert effects; however, the over production of BDNF in vivo does not increase circulating levels in rats (Matthews et al. 2009). As well, serum and muscle BDNF responses are temporally uncoupled following acute exercise in humans, supporting the findings in animals that skeletal muscle is not a source of circulating BDNF with acute exercise (Matthews et al. 2009).

Interestingly, recent work shows that exercising skeletal muscle may influence the expression of hippocampal BDNF via organ cross-talk (Wrann et al. 2013). Specifically, muscle contraction results in the cleavage fibronectin type III domain-containing protein 5 (FNDC5), a sarcolemmal protein, which is then secreted into the circulation as irisin. Irisin is purported to stimulate the expression of BDNF in the hippocampus via the peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α) pathway (Wrann et al. 2013). This mechanism, however, cannot explain the rapid rise in circulating BDNF that accompanies acute exercise given that upregulation of BDNF messenger RNA (mRNA) takes ≥3 h (Matthews et al. 2009; Rasmussen et al. 2009). As such, skeletal muscle is likely not a source of increased circulating BDNF during exercise, but may indirectly increase hippocampal BDNF expression thereby contributing to the long-term improvements in brain structure and function.

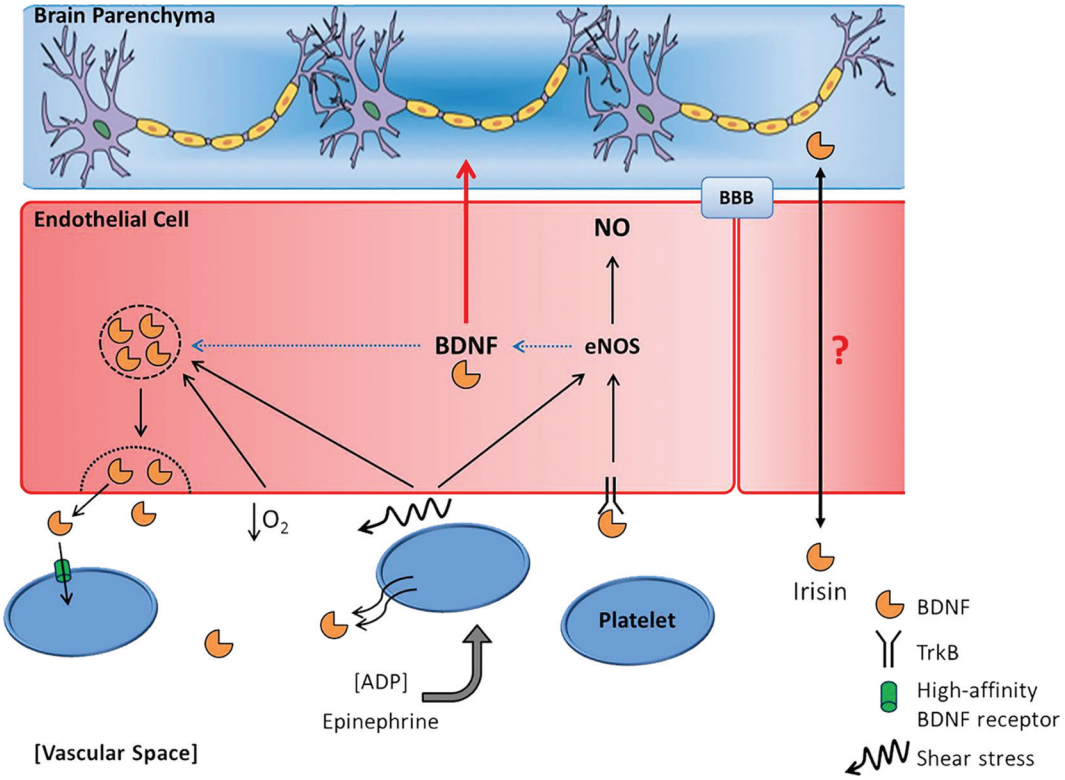
Peripheral blood mononuclear cells

In addition to its neurogenic effects, BDNF also has a role in immunity and tissue repair. Accordingly, PBMCs express BDNF in response to physiological stress such as inflammation (Kerschensteiner et al. 1999) and exercise (Brunelli et al. 2012). It is proposed that these immune cells release BDNF at the site of an injury to facilitate tissue repair and remodelling (Kerschensteiner et al. 1999); however, at present it is unknown if PBMCs contribute to circulating BDNF with exercise. Despite this, PBMCs represent a possible source of exercise-induced BDNF given that circulating PBMCs increase with acute exercise (Brunelli et al. 2012); therefore, future studies should attempt to elucidate the possibility of BDNF secretion from these immune cells.

Vascular endothelial cells

The vascular endothelium (as a unit) is a significant candidate source of de novo circulating BDNF during exercise (Prigent-Tessier et al. 2013; Helan et al. 2014; Monnier et al. 2017). Endothelial cells rapidly secrete BDNF in proportion to the magnitude of exercise-like stimuli, including shear stress (Prigent-Tessier et al. 2013) and reductions in PO_2 (Helan et al. 2014) (Fig. 2). Endothelial BDNF is secreted into the circulation (Guo et al. 2008) and on vascular smooth muscle where it exerts vasorelaxant effects (Prigent-Tessier et al. 2013). The rapid time course of BDNF release in response to acute stimuli suggests a nongenomic mechanism, implicating the production and storage of BDNF by endothelial cells for activity-dependent release (Prigent-Tessier et al. 2013). BDNF protein content in cardiac and aortic endothelial cells mirror hippocampal BDNF con-

Fig. 2. Schematic of the interactions between peripheral BDNF (bound and free), exercise-dependent stimuli, and endothelial cells of the cerebral microvasculature. From right to left: Unbound, circulating BDNF accesses neural tissue by directly crossing the BBB, and the myokine irisin may enter the brain and stimulate hippocampal BDNF expression (indicated by question mark). BDNF can also cross the BBB from the brain to the blood and increase circulating levels. Unbound circulating BDNF also acts on the TrkB receptor on the vascular endothelium, which stimulates the upregulation of BDNF production via eNOS activity. Newly formed endothelial BDNF has 2 plausible fates: (*i*) enter the brain via neurotrophic coupling, or (*ii*) be released into circulation via exocytosis in response to exercise-like stimuli, including shear stress and reductions in PO_2. Platelets release BDNF in response to shear stress and agonist stimuli including ADP, thrombin, collagen, and epinephrine, which increases the local concentration and therefore, the likelihood of circulating BDNF interacting with the TrkB receptor. Platelets also sequester BDNF via very-high-affinity and moderate-affinity receptors in the absence of these stimuli. ADP, adenosine diphosphate, BBB, blood-brain barrier; BDNF, brain-derived neurotrophic factor; eNOS, endothelial nitric oxide synthase; TrkB, tropomyosin-related kinase B. [Colour online.]



tent (Quirié et al. 2012; Prigent-Tessier et al. 2013), and recently it was discovered that removal of the endothelium from the cerebral microvasculature significantly reduces BDNF levels in the hippocampus and cortex of rats (Monnier et al. 2017). This is a major finding that challenges the neuro-centric origin of BDNF and supports the notion that the role of the vasculature extends beyond the matching of blood flow to neuronal metabolic demand (neurovascular coupling), but also provides direct neurotrophic support in the form of secreted BDNF and other neurotrophins to metabolically active neural tissue (neurotrophic coupling) (Guo et al. 2008; Monnier et al. 2017).

Endothelial cells prominently express TrkB receptors and BDNF signalling appears to function as a positive autoregulatory loop (Fig. 2). Activation of TrkB by BDNF increases the activity of endothelial nitric oxide synthase (eNOS), stimulating the rapid production of nitric oxide in a dose-dependent manner (Meuchel et al. 2011), and BDNF production in the cerebral microvasculature is dependent on eNOS activity (Monnier et al. 2017). Therefore, TrkB activation by circulating BDNF increases the production of endothelial BDNF, which may have implications for brain and circulating levels, as well as overall cerebrovascular health.

Endothelial function is sensitive to behavioural and pathological states, and endothelial function directly impacts BDNF expression such that hypertension significantly impairs endothelial BDNF production (Prigent-Tessier et al. 2013; Monnier et al. 2017). Conversely, 7 consecutive days of 30 min/day treadmill running significantly increases BDNF production and release in both the peripheral (Prigent-Tessier et al. 2013) and cerebral vasculature of hypertensive rats (Quirié et al. 2012; Monnier et al. 2017). These benefits may be due to shear stress per se, as chronic exposure to shear stress in vitro upregulates endothelial BDNF to the same degree as exercise training (Prigent-Tessier et al. 2013). Given the ubiquitous distribution of endothelial cells in the cardiovascular system and that shear stress and reductions in blood PO_2 are the primary stimuli for its production and release – conditions that are omnipresent during exercise – the vascular endothelium may be a significant cellular source that contributes de novo BDNF during exercise.

Summary of plasma sources

Of the tissue sources identified above, current evidence suggests the brain and vascular endothelial cells provide the greatest contribution to circulating BDNF during exercise. The time course of secretion from both is consistent with the appearance of circulating BDNF during exercise (Dinoff et al. 2017) and the mechanisms of secretion are omnipresent during exercise. However, while the extant literature recognizes the brain's contribution, researchers are seemingly less aware and/or focused on the vascular contribution. Given the established link between vascular function and brain function (Novak and Hajjar 2010), targeting the vascular endothelium through exercise and nonexercise modalities (i.e., heat therapy) could be effective for increasing circulating

BDNF. Accordingly, more studies are needed to understand the vascular contribution to circulating BDNF in humans and to potentially establish the relative contribution of the brain and the vasculature.

Sources of serum BDNF

Platelets

The aforementioned tissue sources contribute to the serum pool by adding directly to plasma, given that serum measures quantify the combined plasma (unbound) and platelet (bound) BDNF pools (Fig. 1). By far the greater portion of serum BDNF comes from platelets, as they are the primary transporter of BDNF in the blood and contain 99% of total blood-borne BDNF. Importantly, there are extremely low levels of BDNF mRNA in platelets, suggesting that they do not endogenously produce the neurotrophin (Fujimura et al. 2002) (see Serra-Millàs (2016) for a comprehensive review on BDNF and platelets). Recently, the progenitors of platelets, megakaryocytes, have been shown to contain high levels of BDNF, which has been interpreted as the main source of platelet BDNF (Chacón-Fernández et al. 2016). However, megakaryocytes are unlikely the sole source of platelet BDNF as washed platelets rapidly internalize exogenous BDNF through very high- and moderate-affinity binding sites (Fujimura et al. 2002), suggesting that platelet-bound BDNF is derived from both megakaryocytes and sequestered in the blood from cellular sources (Fig. 2).

The role of platelets in BDNF dynamics is often viewed as purely a storage compartment and is overlooked in the context of increasing BDNF via exercise for improving brain health. However, platelets not only sequester and store the majority of circulating BDNF, they also rapidly release BDNF in a dose–response manner to both pharmacological (antidepressants) (Türck and Frizzo 2015) and physiological stimuli (i.e., shear stress and agonist stimuli) (Fujimura et al. 2002) (Fig. 2). Thus, platelets likely play an active role in the release of BDNF into the plasma during exercise, given that 16% of platelet bound BDNF is released under conditions of low shear stress and 32% under high shear stress (Fujimura et al. 2002). The vasculature of active muscle and the brain experience significant increases in blood flow and shear stress during exercise, implicating these tissue beds as probable sites for BDNF offloading from platelets (Smith et al. 2017). Therefore, the sensitivity of platelets to exercise-like stimuli implicate serum as not simply an inert reservoir for BDNF, but rather by extension of platelet-BDNF dynamics, a contributing factor to the bioavailable pool of BDNF.

Splenic storage and release of platelets (thrombocytosis)

At rest, the red pulp of the spleen stores 30% of the body's platelets in a pool that is freely exchangeable with circulating platelets (Wadenvik and Kutti 1988; Chamberlain et al. 1990). Pools of splenic and circulating platelets exists in a dynamic equilibrium such that α-adrenergic stimulation via catecholamines and sympathetic nerve activity causes the release of platelets from the spleen (thrombocytosis) and β-agonists stimulate platelet reuptake (Thoenen et al. 1964; Chamberlain et al. 1990; Bakovic et al. 2013). The spleen is encapsulated by a sheath of collagen fibres, smooth muscle cells, and a tight mesh of elastic fibres and has the capacity to actively constrict in response to sympathetic activation (Benter et al. 2011). Splenic volume is also highly dependent on splenic blood flow and therefore adrenergic modulation of splenic artery tone directly affects splenic volume (Wadenvik and Kutti 1988).

Not surprisingly, exercise increases circulating platelets in an intensity-dependent manner (exercise-induced thrombocytosis) through mechanisms of increased sympathetic nerve activity (SNA) and circulating catecholamines (Gimenez et al. 1986; Stewart et al. 2003; Hulmi et al. 2010). Following cessation from exercise, platelets are re-sequestered by the spleen (Hulmi et al. 2010), with a time course that parallels reductions in serum BDNF following a bout of exercise (Matthews et al. 2009; Yarrow et al. 2010; Walsh et al. 2016) (Fig. 1). Accordingly, thrombocytosis presents as a strong candidate for the exercise-induced increase in serum BDNF as platelets are the primary carrier of blood-borne BDNF and their temporal responses to exercise are tightly aligned (Table 1; Fig. 1).

Acute exercise upregulates the entire BDNF system

While numerous studies have demonstrated that acute exercise increases levels of both plasma (Rasmussen et al. 2009; Seifert et al. 2010; Gilder et al. 2014; Church et al. 2016) and serum BDNF (Ferris et al. 2007; Matthews et al. 2009; Yarrow et al. 2010; Walsh et al. 2016), only 1 study to date has investigated the response of the entire circulating BDNF system – serum, plasma, platelets, and BDNF per platelet. Cho et al. (2012) found that progressive, maximal treadmill exercise increases serum and plasma BDNF, which was accompanied by an increase in platelet count and the amount of BDNF per platelet. This suggests the possible contribution from cellular source(s) and/or from splenic platelets that contain higher concentrations of BDNF.

While the relative contribution of platelets and tissue sources cannot be isolated, the increase in BDNF per platelet likely represents cellular sources rather than from splenic platelets with a higher BDNF content than circulating platelets. The spleen is constantly exchanging circulating and stored platelets, and these pools do not differ in age or cellular content (Chamberlain et al. 1990), suggesting that the addition of platelets via thrombocytosis would not contain higher amounts of BDNF. However, since the release of BDNF by platelets can be altered by conditions such as allergic airway inflammation (Lommatzsch et al. 2005), it is possible that acute exercise impacts the release of BDNF by platelets. Currently, the ways in which exercise impacts bound versus unbound BDNF and the relative contribution of candidate sources of BDNF is poorly understood and requires focused attention on understanding this relationship.

Part 2 – Application of mechanisms for exercise interventions

Acute exercise, BDNF doses, and multimodal training

The teleological nature of the transient BDNF response to acute exercise is hypothesized to represent a "dose" of BDNF that initiates a cascade of neuronal responses that prime the brain for learning and neuroplasticity (Cirulli et al. 2004; Rasmussen et al. 2009; D'Amore et al. 2013; Korol et al. 2013; Bechara et al. 2014; Piepmeier and Etnier 2015). With accumulated bouts of exercise, the repeated exposures to doses of BDNF would be expected to elicit functional and structural adaptations in the brain (Berchtold et al. 2005; Zagrebelsky and Korte 2014).

This proposition strengthens the importance of focusing attention on the mechanisms of BDNF release, as the time period during/following the provision of a BDNF dose represent a window of potentiated neural function and receptivity of the brain to cognitive challenges. We have previously proposed that increasing circulating BDNF via exercise could be used strategically to maximize long-term improvements in cognitive function and neuroplasticity when applied to a multimodal training intervention (Walsh et al. 2016). Specifically, performing cognitively demanding activities such as a learning task or cognitive training when circulating BDNF is elevated would augment the delivery of BDNF to active regions of the brain. This would allow for the delivery of circulating BDNF to be deliberately targeted to regions of the brain involved in facilitation of a cognitively demanding task via mechanisms of neurovascular coupling. Moreover, the potentiation of cognitive function following acute exercise (Walsh et al. 2018) may improve task performance independent of BDNF and may act as an adjuvant for neurovascular coupling and consequently, delivery of BDNF to the brain. Therefore, understanding the sources and mechanisms that contribute to circulating BDNF during exercise can help to maximize an individual's exposure to

acute doses of BDNF over the course of an exercise intervention and potentially maximize brain plasticity.

Target the spleen – a 1-armed example

The contribution of the splenic platelets to serum BDNF has important implications for exercise interventions that are designed to increase blood-borne BDNF. Recently, we undertook a study that tested the hypothesis that an exercise protocol capable of evoking thrombocytosis should be capable of increasing serum BDNF (Walsh et al. 2017). Given that thrombocytosis occurs with increased sympathetic activation, any intervention that can stimulate this mechanism should be able to evoke increases in serum BDNF, independent of total metabolic cost or the size of muscle mass involved (Walsh et al. 2017). Small muscle mass exercise, such as forearm handgrip exercise can substantially increase sympathetic activation (Joyner et al. 1992) and very brief, sustained handgrip exercise (maximal voluntary contraction) causes splenic constriction and a small thrombocytosis (Frances et al. 2008). Accordingly, we hypothesized that forearm handgrip exercise should be capable of increasing serum BDNF through mechanisms of increased platelets due to thrombocytosis.

To test this hypothesis, we had participants perform 10 min of maximal effort and 30 min of submaximal effort forearm handgrip exercise and measured serum BDNF and platelets (plasma determination was unsuccessful; see Walsh et al. 2017). We found that forearm handgrip exercise increased circulating platelets in an intensity-dependent manner, and this was accompanied by a substantial increase in serum BDNF, although an exercise intensity effect was not statistically significant ($p = 0.06$). The reason for the disconnect between platelet and serum exercise intensity responses may be explained by the observation of an increase in the amount of BDNF per platelet with exercise, which may indicate the contribution of de novo BDNF from cellular sources. Interestingly, BDNF per platelet was not different between exercise conditions, which may suggest that either the contribution of BDNF from tissue sources is not affected by handgrip exercise intensity or that the carrying capacity of platelets for BDNF was reached. Nevertheless, the findings of this study suggest that by focusing on the spleen, an individual can effectively achieve increases in circulating BDNF without a whole-body effort, making it a potentially viable exercise modality for increasing circulating BDNF in individuals with limited mobility.

Shear stress elevation for endothelial release of BDNF

The common physiological stress that stimulates the release of BDNF from the platelets and vascular endothelial cells is shear stress. Shear stress is the frictional force exerted by blood constituents on vascular endothelium and is one of the primary stressors that contributes to the positive modulation of the cardiovascular system with exercise and physical activity (Prigent-Tessier et al. 2013). The brain and active skeletal muscle are among the most energy expensive organs in the body and are consequently major sites of blood flow delivery. An increase in metabolic activity in either tissue bed is met by an immediate and sustained activity-dependent increase in regional blood flow and by extension, shear stress. As a result of heightened metabolic activity during exercise, both circulating platelets and vascular endothelial cells are exposed to increased shear stress, which could impact the release of BDNF.

A recent study provides exciting evidence for the possible contribution of the brain and/or vascular endothelial cells to circulating BDNF independent of exercise. Kojima et al. (2017) found that 20 min of head-out immersion in hot water significantly increases serum BDNF, which supports previous findings that exercise performed in the heat results in significantly greater increases in serum BDNF compared with a normothermic environment (Goekint et al. 2011; Lee et al. 2014). These findings are especially compelling because serum BDNF increased without an accompanying increase in platelets, suggesting exclusive contribution from cellular sources (Watson et al. 2005). The ingenuity of this study presents a model worthy of replication, as it isolates 2 mechanisms known to increase BDNF while eliminating the contribution of platelets from the spleen. Given that we are unable to elucidate the relative contribution of shear stress versus hyperthermia to the increase in BDNF from this study, future studies are tasked with determining the relative contribution of these mechanisms. This raises unique opportunities for strategically targeting the cardiovascular system via exercise and nonexercise modalities for systemically increasing BDNF, and targeting cerebrovascular microcirculation via cognitive activation thereby increasing local shear stress and the delivery of BDNF to active tissue.

Hypotheses worth testing

The bulk of exercise and BDNF research to date has focused on whether exercise increases circulating levels and if there is a relationship between changes in BDNF and cognitive function in response to acute and chronic exercise. While the latter focus is still in its relatively early days, it is clear that exercise increases BDNF (Knaepen et al. 2010; Szuhany et al. 2015; Dinoff et al. 2016, 2017). An important future direction of BDNF research in humans is to isolate the relative contributions of specific mechanisms and sources to further maximize the design of exercise interventions for increasing BDNF. Below are some initial questions that we propose should be answered.

What is the BDNF response to acute exercise in asplenic individuals?

A small proportion of the population are living without spleens because of infection (i.e., splenomegaly) or trauma (Benter et al. 2011). Investigations with these people provide a unique opportunity to isolate the contribution of tissue sources of BDNF in whole-body aerobic exercise. We hypothesize that these individuals would have a blunted BDNF response because of the lack of exercise-induced thrombocytosis and that changes in serum would closely parallel changes in plasma.

How does nonexercise sympathoexcitation affect circulating BDNF?

Sympathoexcitation causes thrombocytosis, which leads to an increase in serum BDNF via platelets; however, this phenomenon has not been studied in isolation. Accordingly, investigations that utilize maneuvers other than exercise to increase SNA would allow for the isolation of the splenic platelet contribution to serum only without addition from cellular sources. We have performed pilot work investigating the ability the cold-pressor test (CPT) and the muscle metaboreflex (MMR) – known SNA maneuvers – to increase serum BDNF. While we were underpowered ($n = 8$) for these investigations, we observed no thrombocytosis with the CPT and a small increase in platelets with MMR that was not statistically significant (2.01%; $p = 0.26$). While there was predictably no response to CPT, serum BDNF increased by 8.5% in response to the MMR test, which trended towards statistical significance ($p = 0.06$). Accordingly, future studies should perform an adequately powered study that can evoke more robust increases in circulating platelets in response to sympathoexcitation protocol.

Is the BDNF response affected by repeated bouts of exercise within a short time frame?

Given that acute exercise increases the amount of BDNF per platelet, the subsequent reuptake of platelets by the spleen following exercise theoretically should contain a higher BDNF content. With that in mind, would a second bout of exercise performed within a short time frame (hours) result in a greater BDNF response compared with the first bout?

What is the relative contribution of BDNF within an active limb?

In the same manner that arteriovenous differences are measured across the brain, the same model can be applied to an exercising limb. The effluent from a deep vein draining an active muscle may contain higher plasma BDNF because of endothelial cell and platelet release in response to high shear stress and reductions in PO_2. Such an analysis would need to measure serum, plasma, and platelets to compare the local versus systemic levels of BDNF during exercise. Measuring arteriovenous differences across a single limb would also allow for the manipulation of specific BDNF-releasing mechanisms, such as shear stress, hyperthermia, and hypoxia. We have previously attempted to use this model using an exercising forearm; however, we were unsuccessful at measuring plasma in our samples and could not make this comparison (Walsh et al. 2017). Nonetheless, isolating and manipulating non-brain sources of BDNF would advance our understanding of these mechanisms.

Conclusions

The mechanisms by which exercise orchestrates structural and functional adaptations in the brain are diverse and not well understood in humans. Despite enthusiasm for the actions of BDNF as a candidate mechanism, previous investigations have primarily focused on whether exercise increases circulating levels rather than how this is achieved. By considering the sources and mechanisms of BDNF release with exercise, researchers and clinical exercise practitioners can strategically design exercise and behavioural interventions that maximize BDNF exposure and delivery to the brain. This research is still in its infancy and will require a concerted, systematic effort to establish the relative contribution of individual tissue sources and to test the hypotheses proposed in this review.

Conflict of interest statement

The authors do not have any conflicts of interest to report.

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