Endothelium-dependent control of cerebrovascular functions through age: exercise for healthy cerebrovascular aging

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Bolduc V, Thorin-Trescases N, Thorin E. Endothelium-dependent control of cerebrovascular functions through age: exercise for healthy cerebrovascular aging. Am J Physiol Heart Circ Physiol 305: H620–H633, 2013. First published June 21, 2013; doi:10.1152/ajpheart.00624.2012.—Cognitive performances are tightly associated with the maximal aerobic exercise capacity, both of which decline with age. The benefits on mental health of regular exercise, which slows the age-dependent decline in maximal aerobic exercise capacity, have been established for centuries.

In addition, the maintenance of an optimal cerebrovascular endothelial function through regular exercise, part of a healthy lifestyle, emerges as one of the key and primary elements of successful brain aging. Physical exercise requires the activation of specific brain areas that trigger a local increase in cerebral blood flow to match neuronal metabolic needs. In this review, we propose three ways by which exercise could maintain the cerebrovascular endothelial function, a premise to a healthy cerebrovascular function and an optimal regulation of cerebral blood flow.

First, exercise increases blood flow locally and increases shear stress temporarily, a known stimulus for endothelial cell maintenance of Akt-dependent expression of endothelial nitric oxide synthase, nitric oxide generation, and the expression of antioxidant defenses. Second, the rise in circulating catecholamines during exercise not only facilitates adequate blood and nutrient delivery by stimulating heart function and mobilizing energy supplies but also enhances endothelial repair mechanisms and angiogenesis. Third, in the long term, regular exercise sustains a low resting heart rate that reduces the mechanical stress imposed to the endothelium of cerebral arteries by the cardiac cycle. Any chronic variation from a healthy environment will perturb metabolism and thus hasten endothelial damage, favoring hypoperfusion and neuronal stress.

Introduction

Physical inactivity is a well-established risk factor for stroke (76, 119), and there is evidence that it also increases dementia (133, 136). On the other hand, cognitive functions are positively associated with the aerobic exercise capacities of healthy individuals, from school age children to the elderly (105, 164, 224). In addition, it has been shown in untrained rats that the intrinsic aerobic capacity determines the risk of developing carotid endothelial dysfunction and elevated glucose, lipids, body fat, and blood pressure (229). In humans, these abnormalities, in concert with inflammation, constitute the metabolic syndrome that has been associated with a two- to fourfold rise in the risk of brain infarction (126, 205), even in patients without diabetes (95). This was confirmed in a recent large case control study performed in 22 countries showing that the main risk factors for stroke are hypertension, current smoking, abdominal obesity, unbalanced diet, and sedentariness (175). It is no longer questionable that physical inactivity is a cause of chronic diseases in general such as type-2 diabetes, coronary artery disease, and cancer (31), but how the aerobic exercise capacity can maintain cerebrovascular health remains an important biological question. The vascular endothelium is stimulated by high shear stress in culture (60, 80), while we observed that lifelong voluntary exercise in mice, which likely induces repeated and acute increases in shear stress, contributes to the maintenance of flow-mediated, endothelium-dependent dilatation in isolated cerebral arteries (139). It is therefore possible that the beneficial effects of exercise on cerebrovascular function through age are partly dependent on its beneficial impact on the endothelium. In this review, we propose three ways to partly explain how exercise could contribute to the maintenance of cerebrovascular endothelial function and contribute to support brain functions (Fig. 1): a regular and repeated exercise activity induces repeated and transitory...
increases in endothelial cerebrovascular shear stress, 2) increases sympathetic activity, and 3) reduces resting heart rate. First, we will review the consequences of aging on the cerebrovascular functions, including endothelial functions.

Vascular Theory of Healthy Cerebral Aging

Cerebral vascular aging is a natural process consequent of the gradual decline in the function of the cerebrovascular endothelial cells (ECs) (63, 69, 139), the “cells that rule them all” (69). Second, aging is characterized by the stiffening of the carotid arteries (92), transmitting the pulsations of peripheral flow to the brain microcirculation and thus favoring pulse wave encephalopathy (179). Importantly, endothelial dysfunction and alteration in the biomechanical properties of the vascular wall appear to synergize in cerebral arteries isolated from 6-mo-old mice with severe dyslipidemia (30). Altogether, carotid stenosis (174) and endothelial dysfunction (157, 174, 176) may perturb cerebral artery hemodynamics and contribute to the decrease in cerebral capillary density (68) already detectable during the fifth decade in healthy humans (5).
Because cerebral blood flow (CBF) is tightly coupled to neuronal metabolic demand (1, 25, 56, 142), the development of chronic brain hypoperfusion together with the increase in inter-capillary distances [for review (38)] promotes progressive hypometabolism (168), chronic blood-brain barrier (BBB) inflammation, and leakiness (232), at a stage where brain cell function is irrevocably compromised. It is therefore likely that the age-related cognitive decline is strongly influenced by the cerebrovascular dysfunction [for review (53–56, 157)].

The brain clearly undergoes atrophy with aging (108, 194, 223), but the origin of neuronal damage and loss is still being debated: is it intrinsic to neurons or is it due to a lower blood perfusion that stresses them? Cerebral hypoperfusion would expose neurons to metabolic stresses, eliciting their demise, a deleterious consequence that has been reported in patients with carotid occlusion (156, 157). It is therefore conceivable that the preservation of a healthy cerebrovascular function, targeting the endothelium, should contribute to not only the maintenance of neuronal migration but also the transmission, neurogenesis, and angiogenesis (44, 69, 88, 203).

**Definition of Healthy Cerebrovascular Function: the Coordinated Regulation of CBF to Meet Neuronal Requirements**

The metabolic needs of the brain are important: neuronal activity consumes 20 and 25% of oxygen and glucose, respectively, for only 2% of total body mass in human (13, 69). These numbers also demonstrate that the large energy requirements of the brain come from aerobic metabolism and suggest that the brain has little energy reserve. For example, a simple mechanical whisker stimulation immediately increases cortical blood flow by 30% in 3-mo-old mice (62), showing that energy supply is essential for neuronal function (53, 109). Mismatch between blood flow and metabolic demands could create astrocyte and neuronal stress, putting their survival at risk: consequently, a healthy cerebrovascular function is characterized by its ability to instantly (within seconds) match neuronal metabolic demands.

Large cerebral arteries and surface cerebral arterioles (pial arteries) contribute to total cerebral resistance much more than other vascular beds (71, 125), demonstrating that CBF regulation starts at their level. These intracranial cerebral arteries, however, lack an external elastic lamina (141, 200) and have attenuated media (200), which may render them highly sensitive to abnormal, chronic rises in blood pressure and pulse pressure as suggested by O’Rourke et al. (179) and Heistad (102); thus, in large to small resistance arteries, the endothelium, which senses flow rate, and vascular smooth muscle cells (VSMCs), which sense pressure, need precise coordination to achieve adequate blood supply to the cerebral arterioles and capillaries. Locally, nearby activated neurons, capillaries and arterioles detect multiple neuron-derived metabolic signals, evoking their dilation (13, 99, 121). Activated neurons either directly or indirectly (with astrocytes serving as relays) stimulate arteriolar smooth muscle cells to induce their dilation. Smooth muscle-dilatory mediators are numerous and include the neurotransmitters themselves [neuron-derived nitric oxide (NO)], astrocyte-derived mediators [NO, prostaglandin E₂, epoxyeicosatrienoic acids, potassium ions (K⁺)], as well as metabolites (lactate, adenosine). The pericyte layer of capillaries contains contractile cells analogous to VSMCs (9, 99, 233), allowing capillaries to constrict, dilate and, thus, adjust their diameter (13, 99, 189). Based on in vitro data performed in brain slices, it is proposed that pericyte-regulated capillary diameter can control blood flow to the brain under the influence of neurotransmitter signaling and vasoactive molecules produced by neurons and astrocytes (189). In vivo, it has been shown by two independent laboratories that pericyte deficiency increases BBB permeability by up-regulating endothelial transcytosis, in direct negative correlation with the density of brain pericytes (10, 51). Blood flow in capillaries can be significantly reduced by the constriction of pericytes induced by the application of thromboxane A₂ (73), demonstrating that their constriction can locally influence flow. Two hours after ischemia-reperfusion-associated oxidative stress, and despite recanalization of the MCA, microvascular flow was significantly reduced because of a persistent pericyte constriction that was prevented by a pretreatment with the superoxide scavenger N-tertbutyl-α-phenylnitrene, suggesting that capillary pericytes are sensitive to oxidative and nitrative stress and can impair reperfusion following ischemia in pathological conditions (233). It seems, however, that pericytes have limited impact on blood flow in physiological conditions during neuronal activity, in contrast to upstream precapillary and penetrating arterioles and surface pial arteries (73). However, more remains to be learned about the role of pericytes in the regulation of CBF (9), but it is clear that activated neurons and astrocytes provide the initial signal for the vasoactive response.

In this scheme, the endothelium is therefore not involved in the initial and local dilatory response evoked by neuronal metabolic demands. This initial dilatory response, however, will create a local drop in resistance creating the driving force for blood flow; depending on the intensity of the neuronal metabolic demand, the endothelium in upstream arterioles up to the pial arteries will sense the flow-induced rise in shear stress, acutely dilate the arterioles allowing for the adequate blood supply from the resistance to the metabolic domain. The endothelium is, therefore, a feed-forward player in CBF control. Shear stress (τ, dyn/cm²), the frictional force per unit area acting on the inner vessel wall, is a function of flow rate (Q, ml/s), blood viscosity (η, 0.009 Poise) and the third power of the inside radius of the artery (r, cm) and can be calculated using Poiseuille’s law:

\[ \tau = 4 \eta \frac{Q}{r^4} \pi (131, 148, 184). \]

Shear stress is relatively constant from the aorta to the capillaries varying only between 10.4 and 26 dyn/cm² (117, 131) averaging 15 dyn/cm². It is important to specify that any local chronic (132) or acute (64) variations in blood flow will alter shear stress that will trigger a change in arterial diameter aiming at normalizing shear stress level. The maintenance of shear stress is a response that is highly dependent on the endothelium (64, 132). Chronically, changes in blood flow in peripheral arteries will unbalance shear stress modifying gene expression, wall structure and endothelial function (128, 148) that will lead within weeks to a new vascular diameter to normalize shear stress and maintain it constant (128). This complex response has been shown to be genetically determined in the mouse carotid artery (127), but its implication in CBF regulation has not been studied. Therefore, a change in vascular diameter is not the only biological effect induced by a chronic variation in shear stress. Acutely, however, a change in...
flow rate will stimulate the release of vasoactive factors modifying arterial diameter within seconds to normalize shear stress (72). Both endothelium-dependent dilations (30, 64, 85, 139) and endothelium-independent constriction (40, 146, 217, 227) or both (84, 170, 213) have been reported in isolated cerebral arteries exposed to changes in shear stress. Endothelium-dependent flow-mediated dilation (FMD) is consistently described in peripheral arteries (123, 130, 198).

The age-related endothelial dysfunction may reflect, or be assimilated to a change in shear stress-sensitivity. The role of endothelial dysfunction in chronic and/or acute CBF maladaptation associated with aging is presupposed but it is difficult to experimentally isolate the endothelial dysfunction, considering the multiple impact of the function of the endothelium on the artery diameter, the wall structure, the permeability and transport of molecules as well as the migration of blood cells through the wall (23, 69).

Role of the endothelium in the regulation of cerebrovascular diameter. Two principal mechanisms, dependent on an intact endothelium—the focus of this review—identified in vitro in isolated cerebral arteries, are responsible for what is commonly called the retrograde conduction of vasodilation (RCVD): 1) the transmission of chemical and electrical signals through gap junctions connecting adjacent ECs and VSMCs (i.e., myo-endothelial junctions) (58, 107, 112, 145, 169) and 2) FMD (81, 112, 170).

Longitudinal spreading of a hyperpolarizing current could mediate cerebral RCVD. The current is initiated by activation of local receptor- or channel-mediated membrane hyperpolarization (58, 107). The increased expression level of gap junctions within the endothelium (145) and inhibition of the conducted dilation response by endothelial impairment suggest that the endothelium rather than VSMCs is the favored conduction pathway in brain arteries (59, 107). The physiological consequence of a local RCVD at the level of penetrating arterioles would be a magnification of the drop in resistance induced by neurovascular coupling; in turn, this would increase shear stress in the upstream pial arteries (109).

In cerebral arteries, in vivo, an acute experimental increase in blood flow leads to a transient increase in shear stress that triggers vascular dilation (81). Shear stress-mediated dilation of larger arteries, such as pial and resistance cerebral arteries, is a coordinated response to neuronal activity that amplifies heightened CBF so that metabolic demands can be met (109). The vascular endothelium is central in this chain of events, called “remote FMD,” since its surface is structurally organized to detect wall shear stress variations (7) and as ECs produce and release several endothelium-derived relaxing factors (EDRFs) (197). Since the first demonstration of FMD in vivo in the basilar artery by Fujii et al. (81), a small number of studies investigated potential vasodilators and ionic mechanisms responsible for this phenomenon. The endothelium appears to be crucial for cerebral FMD: in our hands (64) and those of others (213, 227), cerebral FMD was abrogated in vitro after endothelium removal. NO produced by endothelial NO synthase (eNOS) and additional EDRFs were viewed as contributing factors in the flow response (64, 90, 236). Hydrogen peroxide, produced after eNOS (64) and nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase (185) activation, were identified as key contributors to shear-dependent dilation of rodent cerebral arteries, but not by everyone (236).

Partial abolition of FMD by inhibitors of eNOS (64) or NADPH-oxidase (185) implies the participation of yet other EDRFs. Nonetheless, VSMCs also sense and respond to directly applied shear stress, as it is well known for another mechanical stimulus, the rise in blood pressure (i.e., myogenic response). With the use of a computerized model, minimal, but perhaps not negligible, VSMC exposure to shear stress (1 dyn/cm²) was estimated by funneling flow across internal elastic lamina fenestrations (209) and interstitial flow driven by transmural pressure gradients (226). The physiological relevance of this specific VSMC sensitivity to shear stress is not established.

Flow-mediated constriction (FMC) rather than FMD was also reported in isolated rat cerebral arteries, a response that was mostly endothelium independent (39, 40); similar results were reported in isolated cat MCAs in which FMC was accompanied by smooth muscle cell membrane depolarization (146), and FMC was shown to occur in isolated human cerebral arteries as well (124). In piglet pressurized cerebral arteries, low flow rates induced a constriction, whereas high flow rates induced a dilation, both responses being dependent of an intact endothelium (204). Likewise, in rabbit pressurized isolated arteries, a combination of both FMD and FMC has been reported at different flow rates, but only FMD was abolished by disruption of the EC layer (84, 213). Both FMD and FMC are potential mechanisms that could regulate local CBF on the moment-to-moment basis. The use of isolated cerebral arteries for studying flow-mediated effects has not been conducted in a manner conducive to drawing any physiological conclusion. Clearly, some of these observations are species specific rather than cerebral-specific, and vessel size and initial level of myogenic tone (213, 227) certainly contribute to the experimental outcomes. Furthermore, and for obvious reasons in humans, cerebrovascular shear-dependent responses are very poorly documented (217), and we are far away from elucidating how healthy aging affects the mechanisms underlying shear stress-dependent effects required to match CBF with metabolic demands.

As the cerebral endothelium deteriorates with advancing age, its dilatory properties gradually decline (63, 101, 163). The increased blood flow in the middle cerebral artery (MCA) after infusion of the EROS substrate L-arginine is blunted in older compared with younger human subjects (182), suggesting that eNOS enzymatic activity is impaired. The circulating levels of asymmetric dimethylarginine (ADMA), the endogenous eNOS inhibitor, have been shown to increase with age in humans and could qualify as an independent risk factor of ischemic stroke (173). ADMA is increased in middle-age subjects with silent brain injuries (190) and in elderly (>65 yr of age) with ischemic stroke (234). In patients with Alzheimer’s disease, plasma levels of ADMA increase together with the reduction of those of NO (201). Given that ADMA-induced endothelial dysfunction of cerebral arteries (52) is robust enough to decrease CBF in healthy humans (120), we could acknowledge that ADMA accumulation with age contributes to the development of age-related cerebrovascular disorders. Nevertheless, one study reported that ADMA cerebrospinal fluid concentration decreases with age (113). In addition to a dysfunctional eNOS, a rapid inactivation of NO by reactive oxygen species (ROS) could explain the saturated cerebral vasomotor responses seen in the elderly. Indeed, age-associ-
ated attenuation of cerebral endothelial function can result in increased ROS production (163, 167), NADPH-oxidase-derived ROS could account for the weakened CBF response to whisker stimulation, hypercapnia, and low endothelium-dependent vasodilation in old, healthy mice (186). In addition, the production of vasoconstrictors such as thromboxane A2 increases with age (63) and could compete with the dilatory functions of the endothelium. Both thromboxane A2 and oxidative stress could stimulate pericytes and reduce capillary blood flow with advancing age since pericytes are sensitive to both thromboxane A2 (73) and oxidative nitrative stress (233). The reduction in capillary blood flow induced by endothelial dysfunction could contribute to the drop in resting CBF reported in aging humans by positron emission tomography (1) and phase-contrast magnetic resonance imaging (207) and accelerate the development of neurodegenerative disorders, such as Alzheimer’s disease (100, 214) in susceptible individuals.

Pressure-dependent cerebral artery myogenic tone. Through shear stress and arterial wall innervation, the endothelium competes with myogenic responses to regulate CBF. This well-known phenomenon is described as VSMC depolarization induced by heightened intraluminal pressure (36, 140). In cerebral arteries, myogenic responses contribute to CBF stability, i.e., autoregulation (165, 187), despite changes in systemic blood pressure over a wide range of mean arterial pressures (110, 125). It should be noted that a true plateau of CBF autoregulation may not exist in healthy conditions (196). Kontos and colleagues (125) measured regional blood flow by a hydrogen clearance technique in cats and reported that between 81 and 172 mmHg, there was a 0.37% increase in flow per mmHg increase in blood pressure (125). In this study, small caliber pial arterioles (37 to 59 μm) dilated at mean arterial pressure above 170 mmHg, whereas large cerebral arteries (117 to 174 μm) remained constricted up to a mean arterial pressure of 190 mmHg (125). Given the importance of arterial diameter in blood flow regulation, it is relevant to determine whether the myogenic responses of cerebral VSMCs are affected by healthy aging. Aging has been shown to increase VSMC intracellular calcium sensitivity, resulting in higher tone of rat cerebral resistance arteries (86). In 24-mo-old rats, decreased NO synthase-sensitive signaling was proposed to explain the stronger basal tone seen independently of intracellular calcium elevation (86). However, endothelium removal revealed weaker cerebral tone at 24 mo, indicating that the contractile capacity of VSMCs declines with age (86) and suggests that the endothelium becomes proconstrictive. Studies by Maneen et al. (149, 150) support this hypothesis: peroxynitrite, a ROS formed by the interaction of NO and superoxide, induces F-actin nitrosylation, leading to depolymerization and subsequent loss of myogenic tone. With the consideration that increased superoxide, peroxynitrite, and ROS production in general in the cerebral vasculature have been described in several models of aging (45, 163, 167, 186), myogenic responses to pressure might decrease despite heightened tone.

Human cerebral arteries develop myogenic tone with pressure (212, 225). Based on a review of eight studies that measured dynamic cerebral autoregulation in healthy humans during induced or spontaneous changes in blood pressure, older adults (54 to 75 yr of age) autoregulated CBF as efficiently as younger adults (23 to 30 yr of age) (219). Ten-year follow-up of 10 subjects (starting age 24 to 51 yr) detected a small but significant decrease of 16% in the cerebral autoregulation index (37). Therefore, these results suggest that dynamic autoregulation of CBF is still functional with age, but fast adaptive responses may be delayed. This decline in adaptability of the CBF to a rapid change in blood pressure may be related to a slower myogenic response.

Cerebrovascular wall structure. Because of their plasticity, arterial walls of small resistance arteries, including cerebral, adapt to the environment and aging under the influences of the changes in metabolic, hemodynamic, mechanical, and neurohumoral activities (160). In the peripheral circulation, the caliber of resistance arteries is modified to restore local forces applied to the walls (wall tension and shear stress) and to correct blood flow (220). Alterations in the composition or arrangement of major arterial wall elements, such as collagen, elastin, and VSMCs, transform arterial wall structure and biomechanics (220). Neither age-dependent remodeling of cerebral arteries nor its impact on CBF regulation is well known.

Most of our knowledge on cerebrovascular remodeling is derived from animal models of hypertension, suggesting that large cerebral arteries (>200 μm) stiffen: basilar arteries, posterior cerebral arteries, and the MCA stiffen in hypertensive rats (35, 61, 114, 216). In contrast, our group and others have shown that cerebral artery (>150 μm) and arteriole (<70 μm) distensibility is maintained in mice models of atherosclerosis (29, 30), rat and mouse models of hypertension (21, 22), rat model of ischemia-reperfusion (115), and mice deficient in superoxide dismutase (19). In addition, arterial wall hypertrophy accompanied both hypertension and atherosclerosis. Human posterior cerebral arteries (~2.3 mm) were collected postmortem, and their structures were compared in six young (~42 yr of age) and six aged (~70 yr of age) adults with severe cardiovascular disorders; aging was associated with a thicker intima and media, a loss of VSMCs but an increase in collagen, a severe stiffening and concomitant elastin fiber dysfunction with possible fragmentation and reorganization (77). Lumen diameter reduction and wall hypertrophy are manifestations of remodeling that seem present in healthy aging and in aging in the presence of cardiovascular risks (22, 29, 68, 96, 115).

The integrating role of the endothelium: a regulator of cerebral artery diameter and wall structure. On the basis of our preclinical studies, we have good reasons to believe that cerebral artery wall remodeling and endothelial function are linked mechanistically (29, 30). In peripheral arteries, the circumferential stress generated by the blood pressure and the shear stress created by blood flow friction together
reshape the arterial wall in an attempt to maintain basal mechanical forces (143). It remains unknown how cerebrovascular remodeling, presumably occurring in conjunction with cerebral endothelial dysfunction, affects the brain’s capacity to autoregulate its perfusion throughout aging.

From our point of view, defects (functional, mechanical, and structural) in vessels transporting blood to the brain imply that brain metabolism is disturbed. Depletion of energy and oxygen supplies most likely translates into neuronal energy crisis and, in turn, into neuronal loss, a reduction in essential protein synthesis and the accumulation of cytotoxic by-products of brain activity (53, 54, 111). Simply put, this is how chronic hypoperfusion could translate into cognitive impairment (53, 157). For example, a recent analysis from Aanerud and colleagues (1) showed that resting CBF measured by positron emission tomography gradually declines in most areas of the brain between the age of 21 and 81 yr in healthy volunteers, whereas the fall in CBF was less in the primary motor and sensory area. In addition, this study showed that oxygen extraction fraction increases in the frontal and parietal cortices (but not the primary motor and sensory area) as well as in the temporal cortex, as previously shown by others (154, 183). This observation may be of importance because these areas are vulnerable to neurodegeneration (34). An increased oxygen extraction fraction may be a sign of compromised oxygen delivery to neurons and chronically contribute to the transition from a healthy to an unhealthy brain (1). It is not clear, however, whether age-related brain hypoperfusion is the result of brain atrophy in the elderly (199, 235) or whether brain hypoperfusion due to carotid artery stenosis leads to neuronal crisis and loss (156, 158, 172).

Mechanisms of Exercise-Dependent Maintenance of Cerebrovascular Function

One of the hottest topics in geriatrics in the past few years was the recognition that regular physical exercise promotes healthy aging. The Greek physician Hippocrates wrote 2,400 years ago: “That which is used develops, and that which is not used wastes away…. If there is any deficiency in food or exercise, the body will fall sick.” Likewise, we have been aware for a long time that mental health is dependent on a healthy lifestyle (i.e., mens sana in corpora sano, late first century). Today, a plethora of clinical and epidemiological studies reminds us of the benefits that derive from regular aerobic physical exercise: exercise capacity predicts all causes of mortality (122, 228), and high cardiorespiratory fitness is associated with the maintenance of cognitive functions with age (18). In addition, vigorous exercise (running) strongly reduces the number of years with disabilities (43), and exercise delays, prevents, or ameliorates cognitive impairment (47, 87, 104). In a randomized trial, in elderly patients with subjective memory impairment, physical activity improved cognition during the 18-mo, follow-up period (137). In the elderly with mild cognitive impairment, poor physical performance was associated with brain atrophy (147). Physical frailty is also associated with mild cognitive impairment in older subjects (33). Loss of muscle strength is associated with Alzheimer’s disease in older patients (32). Physical inactivity and obesity increases the incidence of stroke by 60%, which is more than that of coronary artery disease (45%), hypertension (30%), colon cancer (41%), breast cancer (31%), type 2 diabetes (50%), and equivalent to that of osteoporosis (59%) (119). Thus physical aerobic exercise should be considered as a potent therapeutic intervention to preserve cognitive function (4). Nonetheless, the mechanisms by which exercise benefits to the brain and to the cerebral vasculature in particular are not well known.

Many mechanisms have been proposed to explain the broad protective actions of voluntary physical exercise on peripheral arteries [reviewed in Giel et al. (89)] (Fig. 1): a decline in resting heart rate after one year of exercise training (82), a higher expression of antioxidant defenses (83), and a reduced inflammation in combination with increased NO release (70, 97, 195), as well as an increased production of endothelial progenitor cells (135). These data demonstrate that the vascular endothelium is highly sensitive to exercise as we recently reported in mice (139). Besides stimulating the endothelium of the cerebral arteries, chronic physical training is also a good “neuronal exercise” leading to remarkable changes in brain function and neuronal adaptations that have been very well reviewed by Mattson (161). Consistently, exercise in rodents has been reported to stimulate neurogenesis (155) together with brain-derived neurotrophic factor production in the hippocampus (2, 91) and to promote synaptic plasticity (2, 91), improving learning and memory capacity. Although we recognize the importance of these neurobiological mechanisms, the focus of our review is to decipher those associated with exercise-dependent maintenance of cerebrovascular functions that are likely to be important in sustaining neurogenesis and synaptic plasticity. How does protection of arteries by physical training influence the brain, an organ that does not “exercise”? We propose three hypotheses: 1) the shear stress-dependent eNOS activity required to match local CBF with neuronal activity (awareness, balance, concentration, etc.) increases during exercise; 2) exercise heightens sympathetic drive and circulating catecholamines to coordinate blood delivery and energy supply, and thus stimulates endothelial function as well; and 3) exercise reduces resting heart rate which, in turn, decreases mechanical stress on cerebral arteries.

Shear stress-dependent responses of cerebral resistance arteries.

Most studies demonstrate that global CBF is not significantly increased during moderate exercise but increases in distinct areas of the brain (Table 1). This has been shown in numerous animal models following radioactive microsphere injections in dogs (94), miniature swine (78), baboons (106), horses (153), and ponies (152). In rats, treadmill exercise increased blood flow in the cerebral hemispheres and the cerebellum (75). However, in an extensive study by Delp et al. (57) with the use of radioactive microsphere injections in miniature swine, it was reported that total blood flow increases by ~20% at 70% maximal aerobic exercise capacity, from 55 to 68 ml·min⁻¹·100 g⁻¹ (Table 1). The authors also reported local increases in CBF in several subcortical areas involved in the control of locomotion and in the integration of sensory inputs and motor outputs (anterior and dorsal cerebellar vermis) in the maintenance of equilibrium (vestibular nuclei), cardiorespiratory control, and vision (57). Importantly, blood flow increases as a function of exercise intensity in the anterior and dorsal cerebellar vermis and in the vestibular nuclei of miniature swine (57). In healthy subjects, glucose uptake measured by ¹⁸F-D-glucose positron emission tomography increases during aerobic exercise in the prefrontal, sensorimotor,
includes anterior, middle, and occipital gray and white matter area perfused by the anterior cerebral artery, the middle cerebral artery, and occipital area. Brain stem includes midbrain, inferior and superior colliculi, pons, medulla, and pyramidal tract.

During exercise, CBF increased in the dorsal occipital cortex (22%). Brain-derived ECs (15), and it has been proposed by the authors that chronic exercise training, by sustaining eNOS expression and activity, could delay the accumulation of amyloid $\beta$-site APP cleaving enzyme $I$ were observed in brain microvessels (15), while NO supplementation in these mice attenuated them (14).

Exercise training seems, nonetheless, to benefit the brain circulation as a whole. Gertz et al. (88) demonstrated that voluntary exercise in mice in a model of mild brain ischemia produced both short- and long-term effects such as improved cerebral perfusion in the ischemic region and cognitive function through activation of eNOS-dependent angiogenesis and recruitment of endothelial progenitor cells. Similarly, Mayhan’s group (12) studied the effect of exercise training on the damage induced by ischemia (2 h)-reperfusion (24 h) in the brain of diabetic rats: the total infarct volume, spanning through the cortical and subcortical area of the hemisphere, was reduced by a treadmill pretraining protocol of 6 to 8 wk (12). Using healthy wild-type mice, Laufs’ group (67) reported that 3 wk of physical training reduced the size of the lesion induced by the occlusion of the MCA (1 h) after 23 h of reperfusion by $\sim$34% (67). This voluntary running protocol improved the neurological sensory-motor deficit score observed 24 h after the injury in mice, but forced training (treadmill) only tended to improve this score (67). In addition, these two studies showed that 1) the endothelial eNOS-dependent dilatory function was improved by exercise in cerebral arteries in vivo (12) and 2) the benefit of exercise was lost in eNOS$^{−/−}$ mice in vitro (67). Although there are discrepancies, these studies in rats and mice suggest that exercise has a global protective impact on the size of the lesion following ischemia-reperfusion, despite the likely fact that blood flow did not increase in all areas of the brain during exercise. In addition, considerable evidence demonstrates that exercise training directly increases synaptic plasticity (49, 221) and production of neurotrophic factors (3, 49, 222). Circulating factors other than the neurotrophic brain-derived neurotrophic factor may also be involved in the global protective effects of exercise training on the cerebral endothelium. We propose that the rise in catecholamines in the circulation during exercise could also contribute to the global beneficial effects of exercise on the cerebrovascular endothelial function.

**Sympathetic activation.** Although cerebral arteries are innervated (66, 218), sympathetic electrical stimulation do not
induce contractile responses in dogs (215) and does not impact CBF in dogs (218), as well as in humans where cerebrovascular CO₂ responsiveness (assessed by transcranial Doppler ultrasound MCA blood flow velocity) is not altered by baroreflex-induced sympathetic activation (144). Under normal conditions, large cerebral artery postsynaptic adrenergic receptors (ARs) have a low sensitivity to norepinephrine (26, 65, 215). An intravenous injection of norepinephrine in cats induces hypertension at concentrations that do not affect cerebral artery diameter in vivo (125). In addition, unilateral cervical sympathetic denervation in baboons does not affect the breakthrough of the upper limit of autoregulation (~150 mmHg of mean arterial pressure), above which CBF increased by 50% together with a decrease in total cerebrovascular resistance (208).

With the use of radiolabeled microspheres, it was shown that sympathetic stimulation in normotensive cynomolgus monkeys decreased CBF in the cerebellum by 26% (103), whereas sympathetic denervation had no impact on CBF; following hypertension induced by angiotensin II infusion (from 70 to 137 mmHg), sympathetic stimulation decreased CBF but without being magnified by hypertension. In cats, however, neither sympathetic denervation nor sympathetic stimulation increased CBF (103). During severe hypertension induced by angiotensin-II infusion (from 93 to 208 mmHg), however, CBF increased significantly, whereas sympathetic stimulation reduced CBF by 29% and limited hypertension-induced BBB leakage (103). In dogs, as in cats, the same study showed that severe hypertension (from 111 to 231 mmHg) was associated with a large increase in CBF (103). During hypertension, sympathetic stimulation decreased CBF by 9% only, much less than in cats and monkeys (103). In healthy humans, blockade of the α₁-ARs impaired dynamic cerebral autoregulation and attenuated any increases in cerebral vascular tone during moderate dynamic exercise (192). In this study, norepinephrine increased in the plasma from rest (1.8 ± 0.2 pmol/ml) to moderate exercise (3.2 ± 0.3 pmol/ml). Others have shown that phenylephrine infusion increases MCA blood flow velocity indicative of an arterial contraction (181), whereas sympathetic ganglion blockade decreases MCA blood flow velocity (237) in healthy humans. Therefore, there is a very strong species difference in the responsiveness of cerebral vessels to sympathetic stimulation.

Norepinephrine released in the synaptic cleft or the circulation target β- and α-ARs on VSMCs to induce changes in vascular resistance. In the brain, however, circulating catecholamines are believed to mildly stimulate VSMCs as they are retained by the BBB (134) while 4% of circulating norepinephrine is estimated to originate from outside of the BBB area in the brain (166). Importantly, tightness of the BBB is reduced by increases in both cerebral perfusion pressure (27) and circulating interleukin-6 levels (162), two changes occurring during dynamic exercise (188). High-intensity exercise has been found to increase the permeability of the BBB without causing structural damage (16), strongly suggesting that during exercise, circulating factors may become important regulators of cerebrovascular tone and neuronal function. But this still needs to be demonstrated.

For the purpose of this review, it is important to recognize that norepinephrine and epinephrine can act directly on the luminal side of the cerebral endothelium. During physical exercise, circulating catecholamines rise 1.5- to 20-fold depending on the intensity and duration of the activity (78, 191, 192, 238). An increasing number of studies describe circulatingcatecholamines as EC survival factors. In cultured human brain microvascular ECs, propranolol (β₁-AR antagonist) has been shown to interfere with crucial steps of experimental angiogenesis (8). In addition, it was shown that β-blockade with propranolol increases rat mesenteric postcapillary venule endothelial permeability, suggestive of a barrier-stabilizing role for catecholamines (206). Endothelial β₁,₂-ARs are coupled to eNOS phosphorylation and thus, NO bioavailability in cultured ECs (129), pulmonary (17), carotid (46), and mesenteric arteries (74). These data support our hypothesis that the activation of the sympathetic nervous system during exercise (i.e., increased endothelial β-AR stimulation) could participate in the maintenance of vascular functions, which may include the cerebral circulation. Therefore, in theory, chronic blockade of β-AR should be deleterious to the cerebrovascular function. But this has yet to be demonstrated. β-AR antagonists (β-blockers) are widely used for the treatment of hypertension and in patients with coronary artery disease, two major causes of stroke (138, 175). In a recent meta-analysis, it was observed that the use of β-blockers reduces significantly less the incidence of stroke in adult hypertensive patients compared with calcium channel blockers and inhibitors of the renin-angiotensin system (230). In addition, comorbidities such as heart failure can increase the risk of stroke in the elderly treated with β-blockers, and different β-blockers (cardioselective, nonselective, dilatory) may impact on the incidence of stroke differently (11). Therefore, the impact of endothelial β-AR stimulation in the maintenance of cerebrovascular function remains ill defined. Our recent data in mice support this concept by showing that a 3-mo treatment with metoprolol worsened endothelial dysfunction in cerebral arteries isolated from severely dyslipidemic mice (30).

Heart rate reduction. Regular physical exercise leads to the reduction of resting heart rate in young or old healthy humans, or in patients with coronary heart diseases (42, 82, 98, 159, 231). On the other hand, it is known that an elevated resting heart rate (>70 beats/min) is an independent risk factor of cardiovascular diseases and shortened life span (79, 118, 211). Yet, the impact of an elevated resting heart rate on the incidence of stroke is not clear. In an aging patient population with coronary artery disease on optimal therapy including drugs targeting the renin angiotensin system and β-ARs, the incidence of stroke is not greater in patients with an elevated heart rate (79). However, it has been shown that resting heart rate is a risk indicator for mortality in the large cohort of patients (>20,000; > 50 yr of age) of the Prevention Regimen for Effectively avoiding Second Stroke (PRoFESS) study enrolled within 120 days after a noncardioembolic ischemic stroke (28).

In the latter study, a resting heart rate higher than 82 beats/min was associated with a greater decline in cognitive function (>2 points on the mini-mental state examination) between one month after inclusion and the last visit (between 2 and 3 yr follow-up), with a cut-off judged of ~70 beats/min for cognitive decline. It has also been shown in mice that slowing resting heart rate increases brain angiogenesis and reduced the lesion area induced by a transient occlusion of the MCA by ~50% (50). With every heartbeat, pulse pressure engenders mechanical stress on arterial walls. This is far more important in conductions arteries, such as the carotids, since their main role is to...
buffer the propagation of pulse pressure waves toward small downstream arteries to stabilize organ perfusion (177). It has been reported that a progressive increase in heart rate caused by pacing in rats was accompanied by progressive and marked reductions (15 to 43%) in carotid artery compliance and distensibility (151) which could further propagate the blood pressure pulse wave into penetrating brain arteries. As we age, accumulated impacts on the carotids gradually break elastin fibers and damage the endothelium (92). As a result of fatigue, it is expected that stiffer carotids can no longer adequately absorb pulse pressure and upstage their role to upstream cerebral arteries. In addition, elastin fatigue will manifest in cerebral arteries, and because they lack external elastic lamina and adventitia (102, 141), the walls will either weaken or stiffen, depending on unknown arterial structural parameters.

Furthermore, it is hypothesized that the deeper penetration of pulse waves in pial vessels and penetrating cerebral arteries could disrupt the BBB (178, 193). Despite the fact that resting heart rate elevation is not statistically related to the increased mortality rate in elderly subjects with cognitive impairment (41), experimental evidence in animals has shown that pure heart rate reduction using ivabradine prevents carotid stiffness (6), cerebral endothelial dysfunction, and cerebral remodeling (30), while increasing brain capillary density (50). Most likely, by lowering resting heart rate, exercise delays the damage associated with the mechanical stress of heartbeats, and by slowing the age-related stiffening rate of large arteries, it limits the rise in pulse pressure inevitably triggered by “stiff pipes”, preserving downstream cerebral artery endothelial function and maintaining cerebral perfusion.

Challenges and Conclusions

We still do not fully understand how exercise signals the maintenance of cerebrovascular function (via free radicals, neurotransmitters and/or metabolic factors). While it seems never too late for our cardiovascular system to benefit from escaping sedentariness (97, 171, 228), we could question what type/intensity/frequency of exercise best improves longevity, cerebrovascular health, and cognition (93). This contrasts with numerous developments in potential nutrition-derived therapeutics (24, 180), which are believed to have beneficial effects on health and life span independently of physical activity. So far, however, it is the latter part, of a healthy lifestyle, that will keep our brain functioning in the long run by maintaining aerobic capacity and cerebrovascular (including endothelial) function as optimally as possible (Fig. 1).

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AUTHOR CONTRIBUTIONS

V.B. and E.T. analyzed data; V.B. and E.T. interpreted results of experiments; V.B. and E.T. prepared figures; V.B. and E.T. drafted manuscript; V.B., N.T.-T., and E.T. edited and revised manuscript; V.B., N.T.-T., and E.T. approved final version of manuscript; E.T. conception and design of research.

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