Intermittent hypoxia training protects cerebrovascular function in Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is a leading cause of death and disability among older adults. Modifiable vascular risk factors for AD (VRF) include obesity, hypertension, type 2 diabetes mellitus, sleep apnea, and metabolic syndrome. Here, interactions between cerebrovascular function and development of AD are reviewed, as are interventions to improve cerebral blood flow and reduce VRF. Atherosclerosis and small vessel cerebral disease impair metabolic regulation of cerebral blood flow and, along with microvascular rarefaction and altered trans-capillary exchange, create conditions favoring AD development. Although currently there are no definitive therapies for treatment or prevention of AD, reduction of VRFs lowers the risk for cognitive decline. There is increasing evidence that brief repeated exposures to moderate hypoxia, i.e. intermittent hypoxic training (IHT), improve cerebral vascular function and reduce VRFs including systemic hypertension, cardiac arrhythmias, and mental stress. In experimental AD, IHT nearly prevented endothelial dysfunction of both cerebral and extra-cerebral blood vessels, rarefaction of the brain vascular network, and the loss of neurons in the brain cortex. Associated with these vasoprotective effects, IHT improved memory and lessened AD pathology. IHT increases endothelial production of nitric oxide (NO), thereby increasing regional cerebral blood flow and augmenting the vaso- and neuroprotective effects of endothelial NO. On the other hand, in AD excessive production of NO in microglia, astrocytes, and cortical neurons generates neurotoxic peroxynitrite. IHT enhances storage of excessive NO in the form of *S*-nitrosothiols and dinitrosyl iron complexes. Oxidative stress plays a pivotal role in the pathogenesis of AD, and IHT reduces oxidative stress in a number of experimental pathologies. Beneficial effects of IHT in experimental neuropathologies other than AD, including dyscirculatory encephalopathy, ischemic stroke injury, audiogenic epilepsy, spinal cord injury, and alcohol withdrawal stress have also been reported. Further research on the potential benefits of IHT in AD and other brain pathologies is warranted.

Keywords: Alzheimer's disease, brain ischemia, cerebral circulation, cerebrovascular risk factors, cognitive function, intermittent hypoxia training, neurodegeneration, nitric oxide, nitrosative stress, oxidative stress, vasoprotection

Experimental Biology and Medicine **2016; 241: 1351–1363. DOI: 10.1177/1535370216649060**

Introduction

Alzheimer's disease (AD) is characterized by deteriorating executive function, verbal fluency, psychomotor speed, and mental flexibility, which progress along with neurodegeneration to produce severe cognitive impairment, dementia and, eventually, death.[1,2] The histological hallmarks of AD include the extracellular accumulation of two amyloid-β (Aβ) peptides, Aβ1-42 in the form of neuritic ''senile'' plaques in close association with astroglia and Aβ1-40 deposits in the perivascular spaces around cerebral arteries and microvessels, and neurofibrillary tangles of hyper-phosphorylated tau within the cell bodies of neurons.[3–6] The most severe AD pathology is found in the hippocampus, amygdala, and mediotemporal regions, followed by the temporo-parieto-occipital junction. AD also affects the frontal heteromodal cortex, temporal cortex, and the basal nuclei.[7,8] AD is incurable; current interventions at best slow its progression, but can neither arrest nor reverse the neurocognitive impairment.[2]

AD is a looming epidemic, especially in developed societies with extended longevity. In the United States alone, approximately 5.2 million people had dementia in 2010, and that number is expected to increase to 13.2 million by 2050.[8] Worldwide, 36 million people had dementia in 2010; by 2050, it is predicted that a staggering 115–140 million people will have dementia.[9–11] Of these dementia patients, 60–80% have AD, and most of the remainder have a closely related syndrome, vascular dementia. In addition to the toll

on the patients and their loved ones, AD has become the third costliest disease entity in the United States, exceeded only by cardiovascular disease and depression.[11]

A small minority of AD cases is categorized as hereditary or familial AD.[2,12] Familial AD can strike earlier in adulthood, when patients are in their 40s and 50s. Familial AD carries a grim prognosis, with rapid onset and progression of the dementia culminating in death within 2–3 years of the onset of overt symptoms. Idiopathic (sporadic) AD typically afflicts patients later in life, and its incidence mounts beyond age 65. An estimated 40% of Americans aged ≥ 85 years have clinically significant cognitive impairment, of which 75–80% have AD.[13] AD is the fifth-leading cause of death among Americans ≥ 65 years old,[14] and the incidence of AD doubles every five years beyond age 65.[9]

Sporadic AD is associated with a variety of risk factors (Figure 1). Unmodifiable factors include advanced age, expression of the apolipoprotein E4ε (APO-E4ε) isoform, and traumatic brain injury.[13] The APO-E4ε gene has been identified as a major risk factor for AD; indeed, 40–50% of AD patients are homo- or heterozygous carriers of the APO-E4ε allele.[13] In addition, several modifiable AD risk factors have been identified, including cigarette smoking, hypertension, type 2 diabetes mellitus, physical inactivity, dyslipidemia, adiposity, obstructive sleep apnea, and metabolic syndrome.[15,16] Since each of these modifiable risk factors has a cardiovascular component,[17] collectively they comprise vascular risk factors for AD, i.e. VRF.[1,18] It is not surprising that cerebral underperfusion in AD has been reported,[19,20] although the cause-and-effect relationship between cerebrovascular dysfunction and AD is a matter of intense debate.[21]

This review will focus on interactions between cerebrovascular function and development of AD. In particular, we will discuss factors responsible for the depression of cerebral blood flow and its contributions to AD. In this regard, we will review evidence that reducing VRF delays AD or slows its progression. In addition, evidence will be presented that moderate, intermittent hypoxia training (IHT) reduces VRF. Finally, we will examine recent preclinical evidence that IHT directly improves cerebral perfusion and cognitive function in animals with experimental AD. Although there are no reports of IHT treatment of humans with AD, reports showing beneficial effects of IHT in other human brain pathologies will be discussed. This review includes Eastern European studies of IHT that are not generally well known in the West.

Cerebrovascular function and AD

The human brain's intense energy requirements demand continuous delivery of O_2 and metabolic substrates to support ATP production. Like the left ventricular myocardium,[22] the brain has a relatively high perfusion rate and limited oxygen extraction reserve that vary temporally and spatially according to specific sensory inputs and regional activity.[23] To meet changing regional requirements for oxygen and metabolic fuels, the cerebral circulation must adjust its conductance and does so by metabolic autoregulation.[24] In the normal cerebral circulation, regional changes in brain function and associated demand for oxygen are readily met by local arteriolar dilation resulting from cellular release of vasodilatory metabolites, including nitric oxide (NO), carbon monoxide, adenosine, and opioidergic

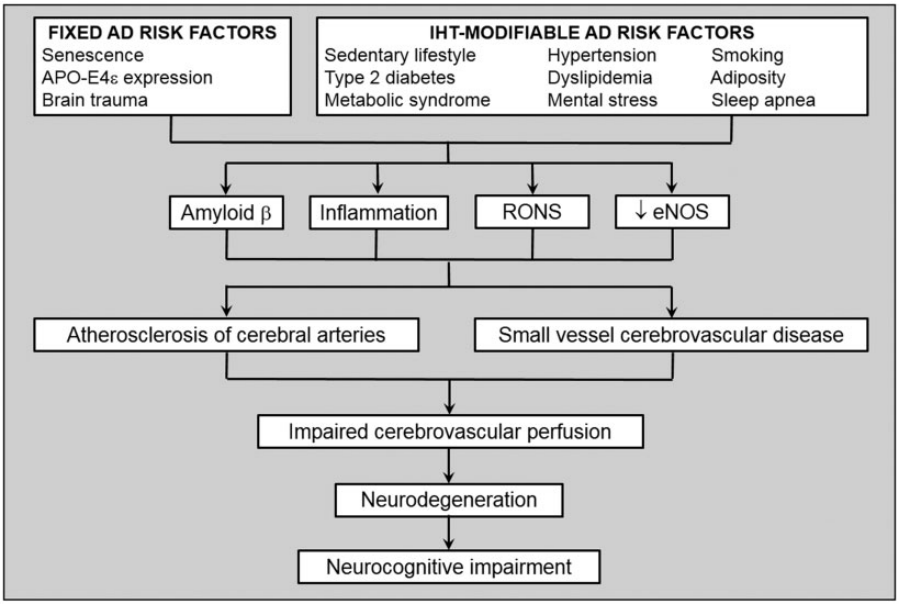

Figure 1 Alzheimer's Disease (AD) risk factors. Risk factors for AD are grouped into those that cannot be modified, i.e. are fixed (upper left) and those that are modifiable by intermittent hypoxia training (IHT) and potentially other interventions (upper right). These fixed and modifiable risk factors give rise to the injury mediators amyloid β, inflammation, reactive oxygen and nitrogen species (RONS), and suppression of endothelial nitric oxide synthase (eNOS) which, in turn, damage macro- and microvascular components of the cerebral circulation. The resultant impairment of cerebral blood supply causes neurodegeneration which culminates in neurocognitive impairment

peptides[25–27] associated with altered tissue PO_2.[28] However, atherosclerotic disease in the cerebral supply arteries limits the vascular reserve that is required for autoregulation of cerebral blood flow and exposes the otherwise healthy, active brain to the potential damage of sustained hypoxia and ischemia.[29–31] The cerebral circulation must also effectively remove metabolic wastes, as well as toxins produced by cerebral disease, most notably Aβ, which accumulates in the brain's perivascular interstitial spaces when cerebrovascular function is compromised.[32] Studies of radiolabeled proteins in rats suggest that pulsations of the cerebral microvasculature secondary to pulsatile flow effect Aβ removal by propelling the peptide along the perivascular interstitium into the lymphatic drainage.[33] Accordingly, Weller *et al*.[33] proposed that vascular stiffening, by dampening pulsatile flow, impairs perivascular Aβ clearance, culminating in cerebral amyloid angiopathy.

In addition to atherosclerotic disease, disorders of cerebral small arteries, arterioles, and capillaries are increasingly recognized as a threat to normal brain oxygenation and function.[34–36] The pathology of small vessel disease includes microvascular rarefaction,[37–39] neurovascular dysfunction,[40] and disruption of the blood–brain barrier.[41,42] It is estimated that 60–90% of AD patients have significant cerebrovascular pathology.[43]

Hainsworth and Markus[44] identified four key clinicopathologic hallmarks of cerebral small vessel disease: small, discrete infarcts within the brain, arteriopathy of small cerebral vessels, diffuse damage to the white matter, and cognitive impairment. An Aβ-mediated positive feedback mechanism amplifies these effects of small vessel disease. With reduced capillary surface area, Aβ clearance is compromised,[45] and elevated interstitial Aβ further depresses endothelial function.[46] Cerebral small vessel disease depresses endothelial synthesis and regulation of NO, and reduced constitutive cerebrovascular production of NO leads to further cerebral hypoperfusion.[47,48] NO also inhibits expression of amyloid precursor protein (APP) and inactivates β-site APP-cleaving enzyme 1, which is involved in formation of Aβ from APP and influences the functional status of microglia.[49]

The net effects of cerebral small vessel disease are impairment of cerebral oxygen and substrate delivery and diminished capacity to remove extracellular respiratory waste products, toxins, and Aβ. Furthermore, vascular-mediated depression of cerebral glucose and oxygen metabolism correlate positively with the severity of AD.[50,51] Moreover, these metabolic disturbances appear to precede neurodegeneration and overt cognitive impairment in AD.[52–54]

Progressive atherosclerotic and small vessel disease chronically limit cerebral oxygen delivery while altering endothelial exchange dynamics. Thus, there is increasing appreciation that vascular therapy might play an important role in slowing or delaying progression of AD. A number of potential interventions for small cerebral vessel pathology have been identified, including neurotrophins, endothelin antagonists, NO donors, prostacyclin mimetics, phosphodiesterase inhibitors, and peroxisome proliferator-activated receptor-γ agonists.[55] However, at present there are no specific therapies approved to improve cerebrovascular perfusion beyond carotid angioplasty,[56] control of systemic arterial blood pressure[57] and blood glucose,[58] and reduction of hypercholesterolemia.[59]

AD is characterized by a cerebral cholinergic deficit.[13] While not specifically directed to the cerebral vasculature, the use of cholinesterase inhibitors to preserve cognitive function in developing AD might indirectly improve cerebral blood flow by enhancing parasympathetic-mediated dilation, or conversely, reduce flow by inactivating the α7-nicotinic acetylcholine (ACh) receptor on extrinsic sympathetic nerves and decreasing NO release.[60] In early AD, cholinesterase inhibitors have been reported to improve cerebral blood flow,[61,62] but negative findings have also been reported.[63] Also, cholinesterase inhibitors increase risk of cardiovascular (bradycardia, heart block, syncope) and gastrointestinal (nausea, vomiting, diarrhea) complications.[64–66] In any case, longitudinal studies of cerebral blood flow are likely confounded by the increasing severity of AD, which, by decreasing neuronal activity, decreases regional metabolism and O_2 demand (Figure 2).

Interventions to treat VRFs

Considering the paucity of direct, effective pharmacological therapies for AD, and in particular for improving cerebrovascular function, other, less direct approaches to delaying or even reversing AD merit study. Studies addressing VRF[1,18,58] have demonstrated significant improvements in AD symptoms and progression.[67] Results of the multicenter randomized clinical trial, FINGER, showed that nutritional guidance, exercise, cognitive training, social activity, and intensive monitoring/management of metabolic/VRFs (e.g. impaired glucose tolerance, obesity, hypertension, hypercholesterolemia) reduced the risk of cognitive decline by 31% compared to a control group receiving only regular health advice.[68] In another study, patients with AD and no evidence of cerebrovascular disease were treated for VRF, including hypertension, dyslipidemia, diabetes mellitus, and smoking, for six months. The rate of cognitive decline of these treated patients was significantly slower than that of untreated patients,[69] although the treatment did not reverse AD.

There is increasing interest in physical exercise to reduce VRF and, thereby, reduce cognitive decline and dementia.[70,71] A meta-analysis of 18 randomized trials showed that aerobic exercise training conferred significant positive benefit on cognition in patients with AD or non-AD dementia.[72] In a recent randomized controlled trial with supervised moderate-to-high intensity exercise, patients with mild AD had reduced neuropsychiatric symptoms.[73] There was a tendency, also, for cognition to improve. Favorable effects of treadmill exercise were also recently reported in a rat model of experimental AD.[74] Physical exercise is well known to increase resting parasympathetic tone[75] and, thus, likely improves resting cerebral blood flow. In addition, exercise has been suggested to promote angiogenesis, neurogenesis, synaptogenesis, and the synthesis of neurotransmitters in structures involved in cognition due to an increase in the liberation of neurotrophic

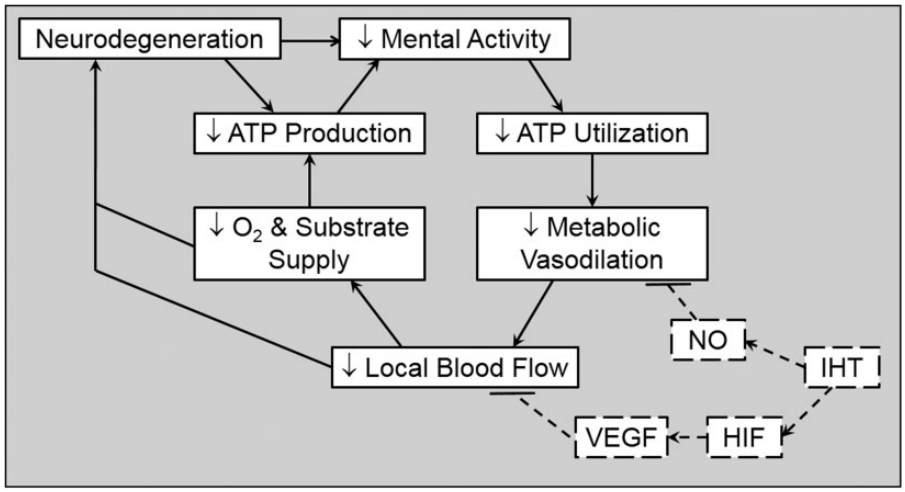

Figure 2 The vicious cycle of dementation and cerebral underperfusion. Decreased activity lowers energy consumption and, thus, demand for blood flow in that brain region. On the other hand, impaired local blood flow, e.g. due to cerebrovascular disease, compromises oxygen and fuel delivery and, thus, ATP production to support brain function. Severe and/or prolonged underperfusion causes neurodegeneration by the mechanisms diagramed in Figure 1. Lower right: the broken lines designate mechanisms mobilized by intermittent hypoxia training (IHT) to acutely increase metabolic vasodilation via nitric oxide (NO) formation, and the more sustained adaptive increase in microvascular density via hypoxia-inducible factor (HIF) induction of vascular endothelial growth factor (VEGF), the principal activator of angiogenesis

factors and the production of enzymatic antioxidants.[71,76] Stimulation of cerebral microvascular NO formation is likely an important means by which exercise increases brain blood flow.[70]

Chronic mental stress is a risk factor for AD.[77–80] Since mental stress has a negative impact on cerebral vascular function,[81–83] and, specifically, increases the risk of cerebral atherosclerosis[84] and vasospasm[85] it may be considered a VRF. Interestingly, meditation provides stress relief and improves cerebral blood flow[86,87] and does so in subjects with memory loss.[88] In addition, meditation reduces other VRF,[89] e.g. by improving blood pressure control, decreasing insulin resistance and reducing lipid peroxidation.[90]

Intermittent hypoxic training (IHT) improves cerebrovascular function and reduces VRF

Characteristics of therapeutic IHT versus adverse hypoxia paradigms

The therapeutic application of moderate IHT reviewed here should not be confused with the intense, episodic intermittent hypoxia associated with sleep apnea, a pathological condition and AD risk factor[91,92] that produces chronic hypertension and has adverse cardiovascular and cerebral effects in AD.[15,16] In contrast, therapeutic IHT utilizes exposures to moderately severe hypoxia produced in hypobaric chambers or by inspiring normobaric atmospheres with O_2 content lowered to approximately 10%. Typical normobaric IHT protocols utilized in our laboratories for human[93–95] and animal[96–100] research involve 5–8 cycles/day of inspiration of 10% O_2 for 5–10 min, with intervening 4 min normoxia (21% O_2), over the course of 14–20 consecutive days. Rats and dogs subjected to these protocols showed no distress, and humans (including coauthor HFD) reported no discomfort. In other laboratories, human subjects with hypertension,[101] ischemic heart disease,[102,103] and chronic obstructive pulmonary disease[104] have completed similar protocols with no untoward effects. When intermittent hypoxia was produced in hypobaric chambers, daily hypoxic exposures were 4 h bouts at a simulated altitude of 4000 m for 14 days. The hypoxic exposures of IHT differ from those of chronic sleep apnea, which are shorter, usually more severe, continue throughout sleep for years, and are often accompanied by the added stress of partial or complete nocturnal arousal and persistent activation of the sympathetic nervous system.[105]

It is well known that chronic hypoxia stimulates angiogenesis in brain and other tissues.[106] However, chronic hypoxia is an impractical means to improve brain vascularity, and this procedure also stimulates significant pulmonary hypertension and right ventricular hypertrophy[107] and increased blood viscosity due to increased hematocrit.[108,109] As might be expected, the duration of continuous hypoxia is critical for development of right ventricular hypertrophy. Recently Ma *et al.*[110] reported that more than three weeks at 3000 m altitude was required for rats to develop pulmonary hypertension, a precursor of right ventricular hypertrophy. Since typical IHT protocols expose subjects to moderate hypoxia intermittently for less than 2 h/day over at most three weeks, the stimulus for pulmonary hypertension and right ventricular hypertrophy should be minimal. In addition, IHT stimulates vasodilatory NO production,[111–113] which should delay the pulmonary hypertensive effects of hypoxia. Thus, it was not surprising that rats treated for 21 consecutive days with IHT had no right ventricular hypertrophy.[100]

IHT improves cerebral blood flow and prevents cerebrovascular rarefaction in AD

In a rat model of experimental AD induced by bilateral injection of a fragment of Aβ (Aβ 25–35) into the basal magnocellular nuclei, cerebral blood flow in the parietal cortex

was assessed by continuous recording with a laser Doppler flowmeter. IHT normalized cerebral blood flow by reducing endothelial dysfunction caused by Aβ[114] and increased the density of blood vessels on the brain surface of rats with experimental hypertension.[115] This finding was more recently extended to a rat model of experimental AD, in which vascular density was significantly decreased in both hippocampus and cortex by 22–25%.[116] However, vascular density in AD rats treated with IHT did not differ from that of the respective brain areas of normal control rats. It seems likely that IHT-induced angiogenesis and the resulting prevention of brain vascular rarefaction would improve cerebral blood flow and its regional distribution.[117]

Cerebral angiogenesis is initiated in hypoxia by the transcription factor, hypoxia-inducible factor-1 (HIF-1) through activation of genes with promoter regions containing hypoxic response elements, including the vascular endothelial growth factor (VEGF) gene.[118,119] In response to hypoxia, VEGF is concentrated primarily in the end feet of the astrocytes surrounding the capillaries[120] where it is ideally positioned to exert paracrine activation of angiogenesis. Conceivably, these mechanisms were also responsible for the cerebral angiogenesis induced by IHT,[121] but this hypothesis requires further investigation. In any case, it is likely that the resulting increases in cerebral blood flow, oxygen and substrate delivery, and removal of metabolic waste products and toxins produced by IHT-induced cerebral angiogenesis would favorably impact brain function. VEGF is one of several HIF-1-responsive genes which, collectively, increase the cell's ability to withstand periods of severe hypoxia and/or ischemia. For example, HIF-1-driven expression of glycolytic enzymes[122] could increase O_2-independent ATP production, and erythropoietin, long recognized for its pivotal role in hematopoiesis,[123] also can be synthesized within the brain, where it exerts a host of neuroprotective actions.[124]

IHT improves endothelial production of NO

Normally, NO involved in cerebral blood flow regulation is produced by endothelial cells, neurons, and nitrergic perivascular nerves.[125] In addition to regulating cerebral blood flow, endothelial NO is vaso- and neuroprotective due to its antiplatelet, antithrombotic, antiproliferative, and antiatherosclerotic functions.[126] Endothelial dysfunction resulting from decreased NO production and bioavailability increases the risk of cerebral underperfusion in AD.[127–129] Since aging-related neurodegeneration can be delayed by stimulation of endothelial NO synthesis,[130] interventions to prevent underproduction of endothelial NO should be valuable in prevention and treatment of AD.

Experimental AD induced by Aβ injection into the basal magnocellular nuclei in rats was associated with attenuated systemic production of NO as indicated by decreased plasma nitrite + nitrate, i.e. NOx.[131] IHT moderately increased NO synthesis and alleviated the decrease in plasma NOx in experimental AD. The reverse was observed when NO production was limited by daily administration of Nω-nitro-*L*-arginine (*L*-NNA), a nitric oxide synthase (NOS) inhibitor, and memory loss was exacerbated. In contrast, increasing plasma NO by administration of the NO donor, dinitrosyl iron complex, prevented the Aβ-induced memory impairment, similar to the effect of IHT. Experimental AD attenuated endothelium-dependent aortic vasodilation, but this effect was not altered by IHT (unpublished observation).

In rats with experimental AD, ACh was injected into a carotid artery, to assess endothelium-dependent modulation of cerebral blood flow, which was monitored continuously with a laser Doppler flowmeter.[114] Endothelium-dependent cerebral dilation was decreased ~77% in experimental AD. Intermittent hypobaric hypoxia (4 h/day for 14 days at 4000 m simulated altitude) prevented completely this endothelial dysfunction; thus, IHT normalized ACh-induced vasodilation to that of non-AD controls.

IHT ameliorates overproduction of non-endothelial NO in AD

NO has both beneficial and detrimental actions in AD.[132–134] Endothelial production of NO is compromised in AD, and this is one factor leading to cerebral underperfusion. On the other hand, excessive production of NO in microgia, astrocytes, and cortical neurons has been demonstrated.[133–139] A key event that triggers NO overproduction in AD is induction of inflammation and immune responses by Aβ, which results in iNOS expression in microglia and astrocytes.[133] Another important event is glutamate excitotoxicity, which excessively increases cytosolic Ca^{2+}.[133] The Ca^{2+} influx into postsynaptic neurons activates nNOS, which is co-localized with glutamatergic *N*-methyl-D-aspartate receptors. Together iNOS and nNOS generate toxic levels of NO.[133] In experimental AD, non-endothelial tissue[140] also contributes to excessive NO formation. Excessive NO is toxic to neurons,[139] where it condenses with superoxide to form peroxynitrite,[112] a powerful oxidant that irreversibly damages lipids, DNA, carbohydrates, and proteins, leading to mitochondrial dysfunction and cellular degeneration.[141–145] This nitrative damage occurs early in the progression of AD and may be important for transition from mild cognitive impairment to the severe impairment characteristic of AD.[146]

To examine empirically the association of AD and NO overproduction, we measured the stable NO metabolites, nitrite and nitrate, in rat brain with experimental AD.[143] The results demonstrated that Aβ induced pronounced brain NO overproduction resulting from excessive expression of all three NOS isoforms. IHT completely prevented this NO overproduction in Aβ-injected rats. In these experiments, Aβ injections were associated with accumulation of 3-nitrotyrosine (3-NT), a product of peroxynitrite modification of protein tyrosyl residues, in cortex and in hippocampus. IHT dampened these increases in 3-NT in both brain regions. In this study, we obtained direct evidence of hypoxia-induced neuroprotection by histopathological analysis of the parietotemporal cortex. Damaged neurons were not found in control rats but multiple shrunken, hyperchromic degenerating neurons were present in the parietotemporal cortex of rats injected with Aβ. After IHT, neurons

with these pathomorphological signs were essentially absent. Therefore, although adaptation to hypoxia *per se* moderately increases NO production,[112,131] it prevents cytotoxic NO overproduction in experimental AD and alleviates adverse effects of AD on memory.[143]

NO can be bound into *S*-nitrosothiols and dinitrosyl iron complexes for transport and intracellular storage.[147–149] These NO stores are formed with any increase in NO, so by sequestering excess free NO, these stores can provide protection by buffering NO overproduction. On the other hand, when NO production is deficient, NO stores can be mobilized to provide an additional NO source.[150]

The protective role of NO stores against NO overproduction in experimental AD was investigated in rats.[114] The size of NO stores was evaluated from the ability of *N*-acetylcysteine (NAC) to release vasoactive products from intracellular NO stores, thereby increasing cerebral blood flow.[114,148] To prevent the contribution of *de novo* synthesized NO to the cerebral vasodilatory response, NO stores were estimated in the presence of the NO synthase inhibitor, *L*-NNA. To reveal NO stores, NAC was injected into the carotid artery, and dilation of cerebral blood vessels was evidenced by an increase in flow measured with Doppler flowmetry. Since the size of NO stores correlates closely with NO production,[111] increased NO stores may be regarded as an indirect marker of NO overproduction. Cerebral NO stores were absent in control rats, but NAC administration revealed NO stores in both hypoxia-adapted and Aβ-treated rats.[114] In rats injected with Aβ after IHT adaptation, NO stores were increased compared to non-adapted Aβ-treated rats. IHT increased NO storage capacity as reflected by the maximum amount of NO, which can potentially be bound to and then released from NO stores.[111] This mechanism may underlie the protection of blood vessels against subsequent NO overproduction in rats with experimental AD, as reported by Mashina *et al.*[114]

IHT reduces oxidative stress in AD

Oxidative stress plays a pivotal role in the pathogenesis of AD.[151,152] Aβ deposits and activated microglia and astrocytes are major sources of reactive oxygen species (ROS) in early AD.[153] Among the numerous harmful effects of ROS overproduction, mitochondrial dysfunction contributes to energy imbalance and apoptosis of nerve cells.[154–157] In addition to increased oxidative stress, AD brain is characterized by impaired antioxidant power. The ratio between reduced and oxidized glutathione, a measure of antioxidant power, is decreased in affected brain regions[154] and also in lymphocytes from AD patients.[158,159] Activities of the antioxidant enzymes superoxide dismutase, glutathione peroxidase, and catalase were found to be decreased in hippocampus of rats with experimental AD.[160] Oxidative stress may contribute to formation of neurofibrillary tangles, too. Specifically, $ONOO^-$, the product of NO-superoxide condensation, nitrotyrosinates the glycolytic enzyme triosephosphate isomerase. The modified enzyme then interacts with tau in a manner that promotes tau aggregation, forming neurofibrillary tangles.[161]

There is direct evidence that IHT potentiates antioxidant defense. Increased activities of the antioxidant enzymes, superoxide dismutase, catalase, and glutathione peroxidase, were found in erythrocytes of rats treated with IHT.[162] IHT of older adult (24-month-old) rats increased the resistance of cardiac mitochondria to phenylarsineoxide-induced permeability transition pore opening and decreased myocardial accumulation of ROS.[163] Gonchar and Mankovska[164] and Gonchar *et al.*[165] demonstrated IHT of rats produced by alternating 5 min exposures to moderate, normobaric hypoxia (10% O_2) and 5 min hyperoxia (30% O_2) decreased lipid peroxidation, increased GSH/GSSG and augmented activities of the antioxidant enzymes MnSOD, glutathione peroxidase, glutathione reductase, and NADPH-generating isocitrate dehydrogenase in lung and liver mitochondria, thereby increasing mitochondrial resistance to acute, severe hypoxia. We observed a significant decrease in production of malondialdehyde, a footprint of lipid peroxidation, in the hippocampus of rats with experimental AD after adaptation to intermittent hypobaric hypoxia.[166,167] Also, Kalachev *et al.*[168] reported in human subjects that IHT reduced total pro-oxidant activity and malondialdehyde concentration, and increased the activities of antioxidant enzymes in blood. In another clinical study, IHT increased antioxidant defenses in hypertensive patients with dyscirculatory encephalopathy.[169] Total blood oxidant activity and malondialdehyde were decreased, while superoxide dismutase, catalase, and glutathione peroxidase activities in erythrocytes were increased. Cerebral circulation and short-term memory of these patients were improved.

Regarding the antioxidant mechanism of IHT, we hypothesize that IHT causes intermittent, moderate activation of ROS formation, which occurs during the cyclic alternations of hypoxia and normoxia during the adaptation sessions. These mild, intermittent increases in ROS may induce expression of antioxidant enzymes,[170] most likely by activating the transcription factor Nrf2, which in turn activates expression in astrocytes of a host of antioxidant genes.[171–173] Interestingly, administration of antioxidant NAC prior to each IHT session abolished protective effects of IHT on ischemic myocardium in dogs.[174]

The pineal hormone melatonin is an antioxidant and a free radical scavenger that is deficient in many AD patients. Melatonin also possesses antiamyloidogenic properties.[175] Thus, there is increasing interest in potential benefits of melatonin in AD.[176,177] Hypoxia exposure of Wistar rats steadily increased plasma melatonin activity to a plateau at seven days.[178] To what extent the beneficial actions of IHT in AD are due to increased release of melatonin by the pineal gland remains to be determined.

IHT reduces VRFs

In addition to its direct effects on cerebrovascular function, IHT has been employed to effectively treat conditions associated with VRF, including systemic hypertension, atherogenic changes in the lipid profile, smoking, obesity and metabolic syndrome, ischemic cardiac disease, and psychological stress.[101,102,179,180] While not specifically

focused on VRF, a recent review by Navarrete-Opazo and Mitchell[181] described IHT's effects on the respiratory, cardiovascular, immune, metabolic, skeletal, and nervous systems. The following discussion extends that of Navarrete-Opazo and Mitchell[181] to more specifically address IHT's impact on VRF.

In a clinical study in patients with stage I hypertension, Lyamina *et al.*[101] found that normobaric IHT decreased blood pressure completely to normotensive values in 92% of patients and partially in the other 8%. In 85% of the patients, the antihypertensive effect of IHT persisted for at least three months. These results supported earlier findings with hypobaric IHT.[182,183] Those studies also reported that about 20% of the smoking subjects stopped smoking after IHT. In spontaneously hypertensive rats, IHT slowed development of hypertension and completely prevented development of vascular endothelial dysfunction.[98]

Since the intermittent hypoxia of human sleep apnea is associated with hypertension, it is not surprising that some animal studies of ''intermittent hypoxia'' have shown elevated arterial pressure. However, those intermittent hypoxia protocols were designed to simulate the intense, episodic intermittent hypoxia of sleep apnea, clearly distinct from the IHT that we have found to be antihypertensive. For example, Perim *et al.*[184] recently concluded that intermittent hypoxia increases arterial blood pressure of rats. In their protocol, FIO_2 was reduced to 6% for 30–40 s, followed by 21% O_2 for 5 min, and these hypoxia–reoxygenation cycles were repeated for 8 h/day over 10 days. Clearly the ''dose'' of intermittent hypoxia is critical for determining its effects on blood pressure and, most likely, other outcomes.[181] It also should be noted that obstructive sleep apnea interrupts alveolar ventilation, producing hypercapnea, whereas IHT has the opposite effect. Studies in rats demonstrated that hypercapnia produced by addition of CO_2 to the hypoxia chamber blunted the cardioprotective effects of moderate hypoxia.[185]

In subjects with ischemic heart disease, hypobaric IHT decreased total cholesterol by 7%, increased high-density lipoprotein by 12%, and decreased low-density lipoprotein by 13%.[186] These changes were greatest at three months post-treatment and persisted for six months. An atherogenic index declined by 26% immediately post-treatment and by 37% at three months post-treatment. However, conflicting data were earlier reported from studies in very young rats exposed to IHT,[187] yet the IHT-treated rats had lower serum low-density lipoproteins as adults.

The effect of IHT on obesity and metabolic syndrome was studied in young men aged 17–25 years.[188] After IHT, body weight, fat mass, and body mass index decreased significantly compared to respective values in untreated control subjects. IHT produced similar, favorable responses in postmenopausal women with metabolic syndrome,[189] including reduced body weight, body mass index, systolic and diastolic blood pressures, serum glucose concentration and insulin activity, and total cholesterol. IHT was found to augment the effects of exercise on obesity.[190]

The beneficial effects of adaptation to hypoxia in ischemic cardiac disease have been recognized for many years.[179,180,191] In 1993, Ehrenbourg and Gorbachenkov[102] described IHT treatment of patients with stable angina. Remarkably, IHT decreased the frequency of angina attacks by $56.2 \pm 5.1\%$ in patients with NYHA classes I and II and by $50.7 \pm 6.1\%$ in patients with NYHA classes III and IV. IHT improved the functional class in 55% of patients. Unfortunately these studies were reported in a peer-reviewed journal not readily available to western readers.

In another early study, Lyamina and Pilyavsky[191] described favorable effects of IHT on cardiac rhythm disorders in patients with neurocirculatory dysfunction. In a more recent study, IHT was performed in patients with functional class I–IV angina who were receiving standard drug therapy for ischemic heart disease, including nitrates, β-blockers, and long-acting calcium antagonists.[192] IHT abolished cardiac arrhythmias in 57% of the patients and reduced arrhythmias in the other 43%. The incidence of anginal attacks decreased by 45% after IHT. Also, this use of IHT as an adjuvant to drug therapy appeared to neither suppress myocardial contractility nor impair cardiac conductance. These clinical studies in patients are substantiated by relevant preclinical studies showing antiarrhythmic and myocardial protective effects of IHT in animals.[96,97,100,193,194] Further information on the cardioprotective effects of IHT are available in the recent reviews by Zhang and Zhou[195] and by Serebrovska and Shatilo.[196] Various antistress effects of IHT have been described by IHT pioneer Felix Meerson.[179] He and his co-workers found that IHT prevented stomach ulcers induced by restraint stress and behavioral disorders in rats.[197] Zhu *et al.*[198] reported that IHT produced antidepressant-like effects in rats subjected to a variety of mild stressors, e.g. restraint, forced swim in ice-cold water, and strobe light, and these effects were accompanied by hippocampal neurogenesis. In studies of alcohol withdrawal stress in rats, IHT attenuated oxidative damage to the brain and mitigated behavioral abnormalities.[99,199] In summary, there is robust evidence that IHT can impact many of the major VRF.

While there are no reports of clinical applications of IHT in patients with AD, there has been considerable research in clinical applications of IHT for other brain pathologies, including dyscirculatory encephalopathy[169] and parkinsonism.[200] In their recent review of IHT and neurologic dysfunction,[201] Gonzalez-Rothi *et al.* stated ''it has become clear that brief, repeated presentations of hypoxia [i.e., acute intermittent hypoxia] can boost the efficacy of more traditional therapeutic strategies in certain cases of neurologic dysfunction.'' While Gonzalez-Rothi *et al.* emphasized the effects of IHT on neurorehabilitation after spinal cord injury, they noted that the fundamental principles of IHT are likely to apply to other dyskinesias.

Intermittent hypoxia training has been shown to afford neuroprotection against ischemic stroke injury. Stowe *et al.*[202] found that IHT of male mice provided protection against transient focal stroke that persisted for eight weeks after the IHT program. Postischemic inflammation, endothelial permeability, and leukocyte diapedesis across the blood–brain barrier were lessened. Brain infarct volumes were reduced, but cerebral blood flow during and after ischemia was not altered. These findings extended earlier reports that IHT prevented cortical infarction following

cerebral ischemia–reperfusion.[203,204] In one of these studies,[203] similar reductions in brain infarct volume were afforded by IHT and ischemic preconditioning of the brain. IHT was also observed to prevent impairment of passive avoidance learning and suppression induced by severe brain hypoxia.[205]

Oligodendrocyte fusion with neurons has been observed in the poststroke rat brain.[206] The resulting binucleated neuron would have enhanced functional capability due to its increased number of processes and synapses. This novel means of neuroregeneration may compensate to some extent for the loss of neurons and synapses during the ischemic insult. We recently found that IHT significantly increased the number of cortical binuclear neurons in normal rat brains.[207] To what extent this mechanism contributes to the favorable effects of IHT in models of stroke remains to be determined.

While no effects of IHT in human stroke have been reported, it is interesting to note that ischemic stroke patients who had previously had spontaneous, transient cerebral ischemic attacks had more favorable neurocognitive outcomes than patients without previous transient attacks.[208] Prior transient ischemic attacks of approximately 10 min duration appeared to provide the optimum protective effect.

Intermittent hypoxia training also had positive effects in rats of the KM strain, which are predisposed to audiogenic epilepsy induced by loud noises.[209] Seizures were prevented and the associated subdural hemorrhage was significantly less in IHT than in non-IHT rats. This antiepileptic effect of IHT was confirmed in a recent study by Zhen *et al.*,[210] where IHT reduced the frequency and severity of seizures in rats with pilocarpine-induced epilepsy. This protection was associated with suppression of intracellular calcium overload and inhibition of neuronal apoptosis in the hippocampus. The antiepileptic effect of IHT may also involve upregulation of monocarboxylate transporter 4 expression in the plasma membranes of rat hippocampal astrocytes,[211] an adaptation that could increase the astrocyte's capacity to supply lactate as energy substrate to the adjacent neurons.[212] In patients with encephalopathy,[169] IHT reduced the elevated peak systolic, peak diastolic, and mean blood flow velocities in the middle cerebral artery. Importantly, IHT alleviated headache and improved sleep and memory function of the patients.

Conclusions and perspectives

The potential limitations of IHT intervention for AD must be acknowledged. As reviewed here, many AD patients have advanced cerebrovascular disease, and while IHT may be safe and readily tolerated by persons with intact cerebral circulations, it is not certain if even brief periods of moderate hypoxia would be without untoward effects in senescent patients with low cerebral blood flow or carotid artery stenosis. However, IHT can be used to modify VRF and therefore may be efficacious in patients who have already had predisposing conditions, such as stroke, transient ischemic attacks, diabetic or dyscirculatory encephalopathy, and post-traumatic stress. Although beyond the scope of this review, it also should be noted that IHT is not only vasoprotective but potentially neuroprotective in AD, as it prevents neuronal degeneration and stimulates neurogenesis and neuroregeneration.[166,167,207]

Another important concern is whether or not IHT would be effective in patients with clinically significant dementia. Because the complex, insidious pathogenesis of AD may begin decades before neurocognitive impairment is manifest, the disease is already far advanced by the time overt dementia is detected, and the opportunity to interrupt its progression may have been missed. On the other hand, the recent identification of the APO-E4ε gene as a major risk factor for AD may afford opportunities for effective, early intervention with IHT. As noted above, 40–50% of AD patients carry the APO-E4ε allele.[13] Timely genetic testing could afford early identification of APO-E4ε, when IHT therapy could be initiated in time to slow progression or even prevent development of AD. Such clinical application of IHT as an early intervention for APO-E4ε-associated AD merits investigation.

Authors' contribution: EBM, HFD, XS, and RTM critically reviewed the literature; EBM, HFD, and RTM drafted the manuscript; EBM, HFD, XS, and RTM edited the manuscript; RTM created the figures; EBM, HFD, and XS edited the figures; EBM, HFD, XS, and RTM approved the manuscript for submission to *Experimental Biology and Medicine.*

ACKNOWLEDGEMENTS

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Funding for this work was provided to XS by an Investigator Grant from the Texas Alzheimer's Research and Care Consortium, and a Pilot Trial Award from the University of North Texas Health Science Center's Center for Alzheimer's and Neurodegenerative Disease Research

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