Cerebral Blood Volume and Vasodilation are Independently Diminished by Aging and Hypertension: A Near Infrared Spectroscopy Study

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Abstract

Background: Senescent changes in brain microvascular circulation may cause or contribute to age-related cognitive decline. Such changes are promoted partly by aging, but also by chronic hypertension, a leading treatable cause of cognitive decline.

Objectives: We aimed to non-invasively detect in vivo the senescent changes in brain microvascular circulation associated with age and hypertension, and inquired whether decrements driven by aging would be exacerbated by chronic hypertension.

Methods: In this longitudinal study, absolute near infrared spectroscopy (NIRS) was used to quantify in vivo cerebral blood volume (CBV) and assess the hemodynamic response to a hypercapnic respiratory challenge in normotensive Wistar-Kyoto (WKY) and spontaneous-hypertensive (SHR) rats. The impact of age and hypertension were evaluated by repeating these measurements on the same animals at 4- and 16-months of age.

Results: CBV decreased markedly with age in both strains, from 4.5 ± 0.2 to 2.6 ± 0.1 ml/100g tissue, on average. Chronic hypertension, however, did not significantly exacerbate this age-related decrease in CBV (−48.1 ± 3.7% in WKYs versus −53.3 ± 5.4% in SHRs). In contrast, vasoreactivity was already impaired in the young hypertensive rats (ΔVMR 0.017 ± 0.014 in young SHRs versus 0.042 ± 0.005 in young WKYs) and further worsened by middle-age (ΔVMR 0.011 ± 0.017 middle-aged SHRs).

Conclusion: Whereas a decrease in brain blood volume correlated with age but not hypertension, vasodilatory capacity was diminished due to hypertension but did not appear affected by age alone. The ability of absolute NIRS to distinguish between such senescent changes in brain (micro)vascular circulation in life may allow early detection and intervention to preserve cerebrovascular health with age.

Keywords: Aging, cerebral blood volume, hypertension, near infrared spectroscopy, vasodilation

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INTRODUCTION

Maintaining healthy brain circulation is undoubtedly critical for the preservation of cognitive health in old age. Senescent changes in brain vasculature, including decreased cerebral perfusion [1], increased vessel tortuosity [2], and reduced vascular tone [3] are common features of brain aging and often accompany Alzheimer’s disease and other neurodegenerative diseases [4, 5]. Such vascular changes have been suggested to play a role not only in the progression of cognitive impairment, but also in its initiation as well [1, 6, 7]. Although age alone remains the greatest risk factor for dementia, hypertension in mid- to late-life has strongly been implicated in increased risk for cognitive decline [8–10]. The higher susceptibility of hypertensive subjects for ischemic infarcts and neurodegenerative disorders has been strongly implicated in increased risk for dementia, but in its initiation as well [1, 6, 7]. Although age alone remains the greatest risk factor for dementia, hypertension in mid- to late-life has strongly been implicated in increased risk for cognitive decline [8–10]. The higher susceptibility of hypertensive subjects for ischemic infarcts and neurodegenerative disorders has been strongly implicated in increased risk for dementia, but in its initiation as well [1, 6, 7].

In order to address this question, we aimed to identify and quantify the individual versus combined contributions of age and hypertension to the senescent changes in cerebral microvasculature. In order to address this question, we aimed to identify and quantify the individual versus combined contributions of age and hypertension to the senescent changes in cerebral microvasculature. We further assessed the vascular impact of age and hypertension on vascular reactivity using our customized non-invasive absolute near infrared spectroscopy (NIRS) method for rat brain microvasculature [17]. The method provides in vivo absolute brain tissue concentration of deoxy- and oxy-hemoglobin, reflecting absolute cerebral blood volume, and provides insight into structural and functional changes in brain microvascular circulation in rat. In order to assess the vascular impact of age and hypertension, we monitored normotensive (WKY) and spontaneously hypertensive (SHR) rats at a young age (4-months) and repeated the measurements a year later, at middle-age (16-months). Spontaneously hypertensive rats (SHR), which are normotensive at birth, develop hypertension by the age of 4 months [13, 18, 19], are the most extensively investigated model for hypertension. The total body weights were 350.2 ± 6.3 g at the age of 4 months and 430.9 ± 9.7 g at the age of 16 months. The total body weights were 350.2 ± 6.3 g at the age of 4 months and 430.9 ± 9.7 g at the age of 16 months.

METHODS

Animals and diets

All animal procedures were approved by the Institutional Animal Care and Use Committee of Tufts Medical Center and Jean Mayer USDA Human Nutrition Research Center on Aging (HNRC). Ten young male rats each of Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) were purchased from Charles River, MA at the age of 3 months and kept in a pathogen-free facility at the HNRC. Ten young male rats each of Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) were purchased from Charles River, MA at the age of 3 months and kept in a pathogen-free facility at the HNRC. Ten young male rats each of Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) were purchased from Charles River, MA at the age of 3 months and kept in a pathogen-free facility at the HNRC. Ten young male rats each of Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) were purchased from Charles River, MA at the age of 3 months and kept in a pathogen-free facility at the HNRC throughout the year of study. The rats were provided with chow pellets and water ad libitum. WKY and SHR rats gained weight at the same rate during the 12 months study. The total body weights ± SE for WKY and SHR were respectively 350.2 ± 5.1 g and 355.2 ± 4.9 g at the age of 4 months and 433.5 ± 6.3 g and 430.9 ± 9.7 g at the age of 16 months.

Non-invasive absolute near infrared spectroscopy (NIRS) instrument and measurements

NIRS instrumentation and measurements on rat have been previously described in detail [17]. Briefly, measurements were performed using a commercial frequency-domain tissue spectrometer (OxiplexTS, ISS Inc., Champaign, IL) operating two fiber-coupled laser diodes (wavelengths of 690 and 830 nm) and a collection optical fiber (3 mm in active diameter), coupled with a photomultiplier tube, which was positioned on the rat’s head using a stereotaxic frame (Stoelting Co., Wood Dale, IL) at a fixed point over the cerebellum, about 1.5 mm left of the sagittal line. The two illumination fibers (400 µm-diameter) were positioned at a distance of 1 mm above the scalp to allow a non-contact, drag-free linear scan and prevent coupling during the scanning. The illumination fibers were linearly scanned away from the collection fiber over...
a range of source-detector distances of ~8–11.5 mm (Supplementary Figure 1) using a mechanical linear stage scanning system (Velmex, Inc., Bloomfield, NY). This scan results in an optical path covering a representative portion of the whole brain. Each linear scan (back and forth) was performed in about 5 s, resulting in a 5 s acquisition time per NIRS data point for tissue hemoglobin parameters.

NIRS measurements were performed on WKY and SHR rats at 4 months and 16 months of age. Prior to each measurement session, the rats were anesthetized by 2% inhaled isoflurane and their heads were shaved. The rats were then transferred to the NIRS instrument where a controlled mixture of O2, N2, and CO2 was administered through a nose mask at a constant flow rate of 5 L/min using a computer controlled GSM-3 gas mixer (CWE, Inc., Ardmore, PA) and anesthesia was maintained at 1.5% isoflurane throughout the entire experiment. In order to assure that the rat was able to properly breathe, vital signs including arterial oxygen saturation (SaO2) and heart rate (HR) were monitored by means of a veterinary pulse oximeter (Nonin, Plymouth, MN) from the rat’s foot during entire experiments. All instruments were synchronized and driven through a control interface designed in LabView software (National Instruments, Austin, TX).

NIRS measurements were performed during 1.5 min of baseline period (i.e., 0% fraction of inspired carbon dioxide (FiCO2)), followed by 3 min of transient hypercapnia challenge (5% FiCO2), and 8 min normoxic recovery at 0% FiCO2. A warming blanket was used to maintain the rats’ body temperature during the procedure. Recovery time and behavior following procedure was assured and recorded.

Following NIRS measurements at the age of 16 months, the rats were anesthetized with 5% inhaled isoflurane and euthanized by bilateral thoracotomy. Normotensive (WKYs) and hypertensive (SHRs) rats displayed similar body weights during the 12 month study. At 16 months of age, SHRs’ brains were significantly smaller than the WKY by about 10% (2.0 ± 0.03 g versus 2.22 ± 0.02 g respectively, p < 0.001), in agreement with previous findings [13]. No sign of anemia was found in WKY or SHR groups at the age of 16-months (blood hemoglobin concentrations 13.03 ± 0.45 and 13.61 ± 0.71 g/dL, respectively) and baseline brain tissue oxygen saturation (SO2) was comparable across both strains at both time-points (Table 1). No mortality was seen in the WKY group, whereas three SHRs died spontaneously between ages 14–16 months. Therefore, the sample size in this study for each group was: Young WKY...
Fig. 1. NIRS output for tissue concentration of total hemoglobin [HbT], oxy-hemoglobin [HbO], and deoxy-hemoglobin [Hb] traces recorded in WKY and SHR rats at 4-months of age (young) and a year later (middle-age). Young WKY n = 10; Middle-age WKY n = 10; Young SHR n = 10; Middle-age SHR n = 7.

Absolute tissue hemoglobin concentration and CBV are significantly reduced with age

By repeating NIRS measurements on the same animals at young (4-months) and middle-age (16-months), we first aimed to monitor a possible change with age in tissue hemoglobin concentration [HbT] and derived CBV. Figure 1 illustrates the NIRS results for young and middle-aged rats, showing the time traces of the hemoglobin species during a 1.5 min baseline, followed by a hypercapnic challenge and a final recovery period (see also Supplementary Figure 2). At each age, WKYs and SHRs displayed comparable tissue concentrations for all three hemoglobin species (Table 1). On the other hand, the repeated measures demonstrated a profound and significant age-related decrease in baseline total hemoglobin [HbT] (Table 1), young animals displaying an average of 82.7 ± 2.8 μM [HbT] versus 39.3 ± 1.7 μM at middle-age (p < 0.05). This
Baseline values of total hemoglobin (HbT), oxy-hemoglobin (HbO), deoxy-hemoglobin (Hb), tissue oxygenation level (StO2), and arterial oxygenation level (SaO2) recorded for normotensive (WKY) and hypertensive (SHR) animals at 4- and 16-months of age

<table>
<thead>
<tr>
<th></th>
<th>WKY young</th>
<th>middle-age</th>
<th>SHR young</th>
<th>middle-age</th>
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<tr>
<td>HbT (µM)</td>
<td>80.5 ± 4.5 a</td>
<td>40.8 ± 2.4 b</td>
<td>84.7 ± 3.7 a</td>
<td>37.2 ± 2.2 b</td>
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<tr>
<td>HbO (µM)</td>
<td>45.0 ± 3.0 a</td>
<td>23.7 ± 2.1 b</td>
<td>47.3 ± 4.2 a</td>
<td>20.4 ± 1.9 b</td>
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<tr>
<td>Hb (µM)</td>
<td>35.3 ± 1.9 a</td>
<td>17.1 ± 1.2 b</td>
<td>37.4 ± 1.7 a</td>
<td>16.7 ± 0.9 b</td>
</tr>
<tr>
<td>StO2 (%)</td>
<td>55.8 ± 1.5</td>
<td>57.2 ± 2.4</td>
<td>55.0 ± 3.1</td>
<td>54.6 ± 2.5</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td>98.2 ± 0.2</td>
<td>97.9 ± 0.4</td>
<td>98.5 ± 0.8</td>
<td>98.7 ± 0.3</td>
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*Values ± SE in the same row designated by different letters are significantly different by Tukey’s test p < 0.05.

The decrease in HbT reflecting a significant decrease in CBV with age was consistently found in each animal measured in this study (Fig. 2A). The magnitude of this age-related decrease was comparable in both strains: CBV in WKY decreased from 4.42 ± 0.25 ml/100mg to 2.71 ± 0.17 ml/100mg (reflecting a 37.7 ± 4.3% reduction), whereas in SHR decreased from 4.65 ± 0.20 ml/100mg to 2.36 ± 0.13 ml/100mg (46.2 ± 5.4% reduction) (Fig. 2A). Therefore, in this study, hypertension did not appear to aggravate the age-related decrease in CBV (p=0.12, t-test). Furthermore, we found that within individuals, the magnitude of decrease in baseline CBV at middle-age linearly and positively correlated to the CBV level at young age (Fig. 2B).

In order to assess the effect of age and hypertension on vasodilatory capacity, we subjected the rats to a hypercapnic challenge. The increased fraction of CO2 in the inspired gas during the challenge indeed drove an increase in absolute CBV, reflecting the dilatation of blood vessels (Fig. 1). Young normotensive rats showed the strongest response to the challenge (Fig. 3A), with an average maximal absolute ΔCBV of 0.175 ± 0.03 ml/100 g tissue. When comparing the maximal response to the respiratory challenge (maximal ΔCBV) for each normotensive animals at young and middle-age, this response was found modestly attenuated by 20% in average at 16-month of age (average ΔCBV of 0.121 ± 0.04 ml/100 g tissue) (Fig. 3A).

**Long-term chronic hypertension impairs vasodilation capacity**

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On the other hand, hypertensive animals displayed from an early age an overall tendency for attenuated response to hypercapnia (average ΔCBV of 0.102 ± 0.06 ml/100 g tissue, Fig. 3B) and by middle-age, the mean SHR response was almost abolished (average ΔCBV of 0.02 ± 0.04 ml/100 g tissue). The relative change in CBV (versus baseline CBV) during the hypercapnic respiratory challenge is represented by the Vasomotor Reactivity index (VMR) time course (Fig. 4). Young and middle-age normotensive rats displayed comparable VMR curves, with similar maximal ΔVMR values (0.042 ± 0.005 versus 0.045 ± 0.014, respectively) and slopes (0.032 ± 0.004 versus 0.030 ± 0.011 s⁻¹, respectively). Hypertensive rats displayed, even at a young age, a lower VMR compared to their age-matched controls (ΔVMR 0.017 ± 0.014), which appeared further aggravated at 16-months of age (ΔVMR 0.011 ± 0.017) (Fig. 4). VMR was therefore significantly affected by hypertension (p<0.05) (Fig. 4).

DISCUSSION

Disturbances of brain microvascular circulation have been suggested not only to accompany, but also to precede the clinical manifestation of cognitive impairment [6, 28–31]. While senescent and pathological microvascular remodeling and rarefaction have been described in postmortem brain, their clinical significance in life has been difficult to ascertain. Detecting the first signs of these structural vascular changes in life and identifying their functional hemodynamic effects is a major challenge for the field and a prerequisite for clarifying their neurological and cognitive consequences.

In this longitudinal study, we used a novel adaptation of NIRS technology for cerebrovascular studies in rat, in order to assess the impact of age and hypertension, major risk factors for developing dementia, on cerebral blood volume and vasodilatory capacity. The noninvasive nature of our method and its capacity to provide absolute (quantitative) measurements, allowed us to follow normo- and hypertensive rats from young to middle-age, and to measure absolute cerebral blood volume and tissue oxygenation in-life at each age, where changes in these values should reflect the extent and nature of cerebrovascular remodeling and aging in this model.

The striking detection of a significant age-related drop of ~42% in cerebral blood volume, both strains combined (Fig. 2) underscores the importance of obtaining absolute NIRS measurements. This finding is comparable to those that we and others have reported in recent human NIRS, which have described decreased oxy-hemoglobin concentration with age of
Young SHR; the average response of the group. During the respiratory challenge. Each curve in the graph represents baseline (1.5 min) was used to calculate VMR at each time point.

SHR

ANOVA test (Young WKY ▲, middle-aged WKY △, middle-aged SHR ●, middle-aged SHR □). Age and strain impact on VMR was assessed by calculating individual maximal ∆VMR and VMR slope of each animal in each group and using ANOVA test (Young WKY n = 10, Middle-age WKY n = 10, Young SHR n = 10, Middle-age SHR n = 7). Individual average HbT value at baseline (1.5 min) was used to calculate VMR at each time point during the respiratory challenge. Each curve in the graph represents the average response of the group about 20–30% [32–34]. Recently, Desjardins et al. [35] reported a decrease of 20% in capillary density in aged rats using two-photon laser scanning microscopy. Together, these results are predicted by the histological evidence describing age-related cerebral microvascular rarefaction in aging. Thinning of the cortex has been shown to occur with aging in humans, and evidences suggest this might start already from middle-age. The contribution of such phenomenon to the decrease in whole brain CBV measured by NIRS in this study remains to be clarified.

The hemoglobin oxygenation levels in tissue (StO2) in this study were similar in all groups, and did not appear to be affected by age. This may be compared to our recent findings showing a decrease of about 6% in brain tissue oxygen saturation in aged (75–100 years old) human subjects [34]. Contrary to our prediction, chronic hypertension exerted only a minor effect on the NIRS indication of decrease in blood volume, compared with the major effect of age. Although SHR rats appeared to experience a steeper decline in CBV with age compared to WKY rats in relative terms (46% versus 38%, respectively), hypertension did not result in a significant reduction in tissue hemoglobin concentrations and CBV, in middle-aged SHR compared to WKY rats (Table 1, Fig. 2). It is possible that the dramatic effect of aging on CBV might have dwarfed any subtle effect that chronic hypertension might have had.

Two types of rarefaction have been suggested to occur due to hypertension. The first, functional rarefaction, involves microvessel constriction to the point of non-perfusion whereas structural rarefaction refers to the anatomic absence of vessels [36, 37]. Further studies will be needed in order to discern between these mechanisms and understand their development over time in brain microcirculation.

By further subjecting young and adult rats to a hypercapnic challenge, during which CO2 is added to the breathing gas, we were able to use NIRS to evaluate cerebral vasomotor reactivity, and to quantify this in both relative (% dilatation) and absolute terms (ΔCBV). Being a potent vasodilator, the increase in inspired CO2 evokes an immediate and large increase in CBV (Figs. 1 and 3). The VMR index allows vasodilatory capacity to be evaluated independently of changes in tissue blood volume, by adjusting each group’s response to its baseline CBV. The VMR index was found to be significantly affected by hypertension (Fig. 4). Whereas WKYs displayed similar percent change in CBV during the challenge at both ages (3.92 ± 0.58% versus 4.27 ± 1.36% change at young and middle-age, respectively), the hypertensive animals from a young age showed a muted response (1.90 ± 1.32%), which further dropped at 16-months of age (1.26 ± 1.62%) (Fig. 4). This represented a decline of ~35% in vasodilatory capacity in middle-age SHRs compared to their response at 4-month of age, and was ~70% lower than the middle-age WKYs. It is interesting to note that when looking at absolute cerebral blood volume values (Fig. 3), young SHRs’ response to the challenge resembled the one seen in middle-age normotensive rats by magnitude (ΔCBV = 0.102 ± 0.06 versus 0.121 ± 0.04 ml/100 g tissue, respectively), as well as by rate of CBV increase during the challenge (slope 0.06 ± 0.05 versus 0.08 ± 0.03 ml/s, respectively). The detrimental effect of chronic hypertension was therefore already apparent at the early stages of the disease, and appeared to progress by the time the hypertensive animals reached middle-age. Since a significant age-related change in vasodilatory capacity was not found in the normotensive control group, it is difficult to conclude from this study whether the further deterioration in vasodilatory capacity seen in middle-age SHRs was a consequence of the long-term hyperten-
the apparently different processes that are thought to underpin the independent associations of aging and hypertension with vascular senescent changes. To the extent that non-invasive absolute NIRS has the ability to detect and identify unique features of cerebral (micro)vascular dysfunction early in-life, NIRS may provide an powerful means of understanding the role of these pathologies in the development and prevention of dementia.

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SUPPLEMENTARY MATERIAL

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sion or a hypertension-effect further aggravated by age.

With respect to hypercapnia, our findings with NIRS are generally consistent with findings from MRI, PET, or Doppler ultrasound studies, which have also indicated an age-related decrease in vasoreactivity to CO2 in aged rats [38, 39], in hypertensive rats [40, 41], and in elderly humans [42, 43]. Cerebrovascular hypertrophy (thickening of wall area), vasoconstriction (luminal narrowing), and vascular stiffening (increased collagen content and rigidity of the vessels’ wall) are part of the vascular remodeling in the brain of hypertensive rats [13, 15, 44], and are believed to be the main factors causing impaired reactivity to carbon dioxide [40, 41].

Theoretically, such structural changes associated with long-term hypertension predict functional reductions in cerebral blood volume and therefore blood supply to the tissue that could lead to cognitive impairment [9, 45], however, this has been difficult to ascertain. The effect of hypertension in the animal model of SHRs was not reflected in this study by diminished outcomes in cognitive behavior. Middle-aged SHRs subjected to Morris-Water-Maze (MWM) and Novel Object Recognition (NOR) behavioral tests showed better performances in both tests compared to the WKYs (Supplementary Figure 3B, C). These results are consistent with several reports on behavior in SHR and WKY rats [46, 47] and are attributed to the SHRs’ hyperactive behavior [48], in contrast to the low-activity, anxiety-like behavior seen in the WKY strain (compared to other normotensive strains). The SHRs’ hyperactive behavior, reflected, for example, by a significantly higher swimming speed in the MWM (Supplementary Figure 3A), makes therefore the effect of hypertension in the animal model of SHRs has been debated [46, 49]. Further studies on cognitive decline in this animal model difficult. The use of different rats’ strains as an appropriate control will be needed in order to elucidate a possible correlation between hypertension and an increased risk for cognitive impairment.

In conclusion, by following normo- and hypertensive rats from young to middle-age, we have demonstrated a profound age-related decrease in absolute tissue hemoglobin concentrations and CBV. This age-related decrease was not found to be exacerbated by hypertension in the SHR model. Vasoreactivity, however, was impaired in hypertensive animals already at a young age, but did not appear to be affected by age alone. NIRS allowed us to detect and distinguish between apparently different processes that are thought to underpin the independent associations of aging and hypertension with vascular senescent changes. To the extent that non-invasive absolute NIRS has the ability to detect and identify unique features of cerebral (micro)vascular dysfunction early in-life, NIRS may provide a powerful means of understanding the role of these pathologies in the development and prevention of dementia.


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