Aging is associated with changes to the biomechanical properties of the posterior cerebral artery and parenchymal arterioles

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Running head: Age-associated changes in the cerebral arteries

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Abstract

Artery remodeling, described as a change in artery structure, may be responsible for the increased risk of cardiovascular disease with aging. Although the risk for stroke is known to increase with age, relatively young animals have been used in most stroke studies. Therefore, more information is needed on how aging alters the biomechanical properties of cerebral arteries. Posterior cerebral arteries (PCAs) and parenchymal arterioles (PAs) are important in controlling brain perfusion. We hypothesized that aged (22-24 month old) C57bl/6 mice would have stiffer PCAs and PAs than young (3-5 month old) mice. The biomechanical properties of the PCAs and PAs were assessed by pressure myography. Data are presented as mean ± SEM; young vs old. In the PCA, older mice had increased outer (155.6 ± 3.2 vs 169.9 ± 3.2 μm) and lumen (116.4 ± 3.6 vs 137.1 ± 4.7 μm) diameters. Wall stress (375.6 ± 35.4 vs 504.7 ± 60.0 dynes/cm^2) and artery stiffness (β-coefficient: 5.2 ± 0.3 vs 7.6 ± 0.9) were also increased. However, wall strain (0.8 ± 0.1 vs 0.6 ± 0.1) was reduced with age. In the PAs from old mice, wall thickness (3.9 ± 0.3 vs 5.1 ± 0.2 μm), and area (591.1 ± 95.4 vs 852.8 ± 100 μm^2) were increased while stress (758.1 ± 100.0 vs 587.2 ± 35.1 dynes/cm^2) was reduced. Aging also increased mean arterial and pulse pressures. We conclude that age-associated remodeling occurs in large cerebral arteries and arterioles and may increase the risk of cerebrovascular disease.

New and Noteworthy

Aging is associated with changes to the biomechanical properties of parenchymal arterioles and posterior cerebral arteries; this could compromise cerebrovascular health
and increase the risk of stroke and dementia. Our studies are novel because of the advanced age of the mice studied and the analysis of the parenchymal arterioles.

Keywords

Aging, cerebrovascular circulation, remodeling, vasculature.

Introduction

Aging is characterized by a decline in many physiological and vascular functions (5). Artery dysfunction (23) is an important factor in cardiovascular diseases such as hypertension, atherosclerosis and cerebral artery disease which are major causes of mortality in the elderly (36). The incidence of cardiovascular and cerebrovascular disease increases significantly with age, this is especially true for stroke and heart failure (24, 25). The remodeling of arteries that occurs with age may contribute to this association between age and cardiovascular disease (31). The term “artery remodeling” refers to stable changes in artery diameter and wall structure; inward remodeling is a reduction in lumen diameter while outward remodeling refers to an increase in lumen diameter. Hypertrophic remodeling occurs when wall area is increased, while hypotrophic remodeling is a reduction in wall area (41, 59). Age-related cerebral artery remodeling could increase the risk of cerebrovascular accidents especially in situations where other risk factors, such as hypertension, are present (48). Therefore it is important to fully understand the effects of aging on cerebral artery structure.

The Stroke Treatment Academic Industry Roundtable (60) recommendations for preclinical testing state that potential neuroprotective agents should be tested in aged
animals. The effects of aging on peripheral arteries have been documented (25, 26). Aged atherosclerotic mice exhibit outward remodeling of the aorta compared to young mice (39). Artery stiffness increases with age in the rat aorta and small mesenteric arteries (27, 31). Hypertrophy of the artery wall has also been observed in small mesenteric arteries from aged rats (1, 27, 35). Aging also causes endothelial dysfunction in arteries from different vascular beds. Endothelial function is impaired in aorta, carotid, and basilar arteries from 18 and 24 month old mice (34, 6, 10). Interestingly, the basilar artery had the most impaired function and the authors attributed this to increased reactive oxygen species production and oxidative stress (34, 47). These studies suggest that the effects of aging on the peripheral and cerebral circulation are different; therefore we cannot assume that the effects of aging in the periphery will translate to the brain.

Cerebral artery autoregulation is an important mechanism to maintain cerebral blood flow within a normal range. The effects of aging on autoregulation are controversial. Recent studies in 24 month C57Bl/6 mice show that aging impairs the ability of the cerebral arteries to autoregulate (52). This has also been observed in clinical studies (8). However, others studies suggest aging has no effect on autoregulation. A recent study in elderly people with mild cognitive impairment showed that low blood pressure was not associated with reduced cerebral blood flow (15). This suggests that in these patients autoregulation is normal. Similar findings have also been made in a younger population (54).
Cerebral arterioles interact with neurons, astrocytes, and glial cells to form the neurovascular unit, which coordinates coupling between neural activity and local cerebral flow. Therefore cerebral arteries may behave differently from arteries in the peripheral circulation (28). The goal of our study was to characterize the effects of aging on the biomechanical properties of the PCA and PAs and to assess the differential effects of aging on the microcirculation and the large pial arteries. We hypothesized that aging would impair the biomechanical properties of the PCA and the PAs resulting in outward artery remodeling and increased artery stiffness. The PCA regulates blood flow and pressure to the posterior cerebral circulation. The PCAs arise from the basilar artery and supply the midbrain, basal nucleus, and thalamus, among other structures (22). The PCA is used as a model of a large pial artery. It is frequently studied in mice because it is more amenable to pressure myography studies than the middle cerebral artery (MCA), which is highly branched. The PAs arise from the pial arteries, via the penetrating arterioles, which are located in the Virchow-Robin space. PAs studied were branching off the MCA and were 1-2mm downstream the Virchow-Robin space, these arterioles are in direct contact with the brain parenchyma. Unlike the MCA or PCA, the PAs have few branches. The PAs play a critical role in controlling blood flow and pressure in the cerebral microcirculation and are important in determining overall cerebrovascular resistance (12). PAs are composed of endothelial cells and smooth muscle cells but they are different from pial arteries and arteries in peripheral vascular beds in that they lack extrinsic innervation (9).
Materials and Methods

Animal Model

All experimental protocols were approved by the Michigan State University Animal Care and Use Committee and were performed in accordance with the American Physiological Society’s *Guiding Principles in the Care and Use of Animals*. Male C57Bl/6 mice purchased from the National Institute of Aging at Charles River Laboratories were housed on 12h: 12h light/dark cycle with food and water ad libitum. Mice were studied at three-to five-months (young) and twenty-two to twenty-four-months (old) of age. For pressure myography studies, mice were euthanized by CO$_2$ asphyxiation followed by cervical dislocation and decapitation.

Telemetry

Blood pressure was measured by telemetry as described previously (62). Mice were anesthetized with 3% isoflurane /1% oxygen for implantation of a catheter attached to a radiotelemetry transmitter (Data Sciences International, St Paul, MN) in the abdominal aorta via the femoral artery; the transmitter body was placed subcutaneously. Mice were allowed to recover for 3 days and then mean, systolic and diastolic arterial pressures were measured continuously (10 second averages collected every 10 minutes, 24hrs per day). Data were expressed as the 1h or 24h averages of systolic, diastolic, mean arterial pressure and pulse pressure (systolic pressure – diastolic pressure). We report the latter because it is an independent risk factor for cardiovascular disease.
Pressure Myography

The brain was collected at euthanasia and the biomechanical properties of isolated PCA and PAs were assessed by pressure myography as described previously (42, 43, 44, 45). To dissect the PAs, a 5 x 3mm section of brain containing the MCA was isolated. Then the pia with the MCA was separated from the brain and the PAs branching from the MCA were used for experiments (43). The PAs we studied were branching off the MCA and were located 1-2mm downstream the Virchow-Robin space. PCAs and PAs were mounted between two glass micropipettes in a custom-made cannulation chamber (61). A servo-null system was used to pressurize the arteries and arterioles. Arteries and arterioles were equilibrated in physiological salt solution (PSS) containing 141.9mM NaCl, 4.7mM KCl, 1.12mM KH₂PO₄, 1.7mM MgSO₄·7H₂O, 10mM HEPES, and 5mM Dextrose under zero-flow conditions at 37°C. Ethylene glycol tetraacetic acid (EGTA; 2mM) and sodium nitroprusside (SNP; 10⁻⁵M) were added to maintain the smooth muscle in a relaxed state. A leak-test was performed prior each experiment, any artery that could not maintain its intralumenal pressure (60mmHg for the PCA and 40mmHg for the PAs) was discarded. A pressure-response curve was constructed by increasing the intralumenal pressure from 0-120mmHg at 20mmHg increments. The PCAs and PAs were equilibrated at each pressure for 5 minutes then lumen and outer diameters were measured using a 10X objective (Nikon Plan objective; Numerical Aperture: 0.25) with a Nikon Eclipse TS100 microscope. The average of the outer and lumen diameter at each pressure was recorded using MyoVIEW II 2.0 software (Danish Myo Technology, Aarhus, Denmark). These measures were compared and used to calculate wall thickness (outer diameter – lumen diameter). Wall cross-sectional area was
calculated using the formula “artery area – lumen area”. The wall-to-lumen ratio, wall stress, strain, and distensibility were calculated as described previously (4). Wall stiffness was quantified using the \( \beta \)-coefficient calculated from the individual stress-strain curves using the model \( y = ae^{\beta x} \); \( y \) is wall stress, \( x \) is wall strain, \( a \) is the intercept and \( \beta \) is the slope of the exponential fit; a higher \( \beta \)-coefficient represents a stiffer vessel.

**Immunofluorescence**

Quantification of artery and capillary numbers in young and aged mice was performed by immunofluorescence (IF) staining of the endothelial cell marker isoelectin GS-IB\(_4\). Mice were transcardially perfused with 100 mL of PSS containing 2.8mM calcium plus 1,000UI/mL heparin sodium salt, \( 10^{-4}\)M SNP, and \( 10^{-5}\)M diltiazem to maximally dilate the cerebral vasculature. The perfusion pressure was maintained at 60mmHg. Following perfusion with PSS, mice were perfusion-fixed with 60mL of 4% formaldehyde. Brains were removed and post-fixed in 4% formaldehyde for 48 hours. Brains were then washed twice in 0.01M phosphate buffered saline (PBS), and placed in 20% sucrose-PBS solution for cryosectioning. Cryosections (20\( \mu \)M thick) were incubated overnight in 0.01mg/ml isoelectin GS-IB\(_4\) Alexa Fluo-568 conjugate (Invitrogen, Cambridge, CA) at 4°C. This is a conjugated lectin, therefore incubation with a secondary antibody was not necessary. The next day, sections were washed 4x in 0.01M PBS (5 min each wash) and coverslips were mounted using Prolong antifade reagent (Invitrogen, Carlsbad, CA) (21). Two fields of the premotor cortex, one in each hemisphere, more specifically in the second and third layers of the neocortex were acquired using a 20X objective (UPLSAPO 20X NA: 0.75) coupled to an inverted Olympus Confocal Laser Scanning
microscope (Olympus America Inc, Central Valley, PA) with Olympus Fluoview FV1000 (Olympus America Inc, Central Valley, PA). All images were acquired using the red fluorescent dye Alexa Fluor 568 that has an excitation wavelength of 578nm and an emission wavelength of 603nm. Sections without the isolectin served as negative controls. For the quantification of the vessel density 3D volume reconstruction of the z-stacks were made. We rotated the 3D volume reconstruction to better visualize when a vessel started and where it ended to make sure we were not counting the same vessel twice. We also used a grid to make sure we were counting the vessel correctly and not twice. We did not have software available to do the quantification of the vessels therefore all the quantifications were done manually by the investigator using ImageJ (46).

**Calcium and collagen staining**

The Investigative Histology Laboratory at Michigan State University performed staining for calcium and collagen in the cerebral arteries. Calcification of the intraparenchymal arteries was assessed using the Von Kossa stain (33). Six random fields were acquired to count the number of positive vessels that contained calcium deposits. Masson’s Trichrome stain was used to stain collagen in the cerebral arteries (7). Six fields were acquired to quantify the amount of collagen deposition in the vessels. Images were acquired using an Axioskop 40 (Carl Zeiss, GmbH, Gottingen, Germany) coupled to a camera (AxioCam MRc5 Carl Zeiss Inc.) with the AxioVision Rel 4.6 software (Carl Zeiss Imaging Solutions, GmbH, Gottingen, Germany). A blinded investigator analyzed images.
Statistical Analyses

All data are presented as mean ± SEM. Body weight, blood pressure, calcium and collagen deposition, and vessel quantification data were analyzed by Student’s t-test. For analysis of artery structure, two-way analysis of variance was utilized followed by Bonferroni t-test for post-hoc comparison of the means. All statistical analyses were performed using GraphPad Prism 6.0 software (GraphPad, San Diego, CA). In all cases statistical significance was denoted by p<0.05.

Drugs and Chemicals

All drugs and chemicals were purchased from Sigma Aldrich (St. Louis, MO) unless otherwise stated.

Results

Physiological Measures

Old mice were significantly heavier than the young mice (31.02 ± 1.57 vs 34.79 ± 1.09 g; young vs old). Blood pressure, measured by telemetry, showed that in our cohort of mice, advanced age was associated with higher systolic, diastolic, mean and pulse pressures (Figures 1A-1D). However, we observed no significant effect of age on heart rate or activity (Figure 1E, 1F). The higher blood pressure in older mice resulted mainly from substantial differences in blood pressure during the night-time when the animals are most active (Figure 2).
Biomechanical properties of the posterior cerebral artery and penetrating arterioles

Older mice had increased PCA outer and lumen diameter (Figure 3A, 3B). Lumen cross-sectional area was also larger in the old mice (Figure 3C). No significant differences between young and old mice were observed in the wall thickness and cross-sectional area (Figure 3D, 3E). Older mice showed decreased wall-to-lumen ratio (Figure 3F). The mechanical properties of the arteries differed with age. Wall stress was higher in old mice (Figure 4A) while wall strain, distensibility, stress vs. strain were lower in PCAs from old mice (Figure 4B, 4C, 4D).

Aging was also associated with PA remodeling (Figures 5, 6). Wall thickness, cross-sectional area and wall-to-lumen ratio were larger in PAs from old mice compared to young. No other significant differences in artery structure were observed. Older mice had greater wall stiffness in the PCAs (Figure 7A) but not the PAs (Figure 7B).

We compared arterial stiffening between the PCA and PAs in young and old mice separately. In the young mice, the PAs were stiffer than the PCAs (Figure 7C). No differences in stiffness were observed between the PCAs and PAs from the old mice (Figure 7D).

Calcium and collagen in the arterial wall

In a small cohort of mice, we observed that older mice did not have a significantly greater number of cerebral arteries with increased calcium deposits in the wall (p=0.3)
(Figure 8A). However, as shown in the representative images, it appears that the percentage of calcification in the individual cerebral arteries from old mice is greater (Figure 8C,D). Arteries from old mice had more collagen deposition (Figure 9A). Representative images are shown (Figure 9B,C, D).

**Artery and capillary density in the cerebral cortex**

Artery and capillary density was quantified using the endothelial cell marker Isolectin IB-4. Two fields of the neocortex, one in each hemisphere, were acquired. In a small cohort of mice, we observed that old mice had significantly fewer arteries and capillaries in the cerebral cortex (Figure 10).

**Discussion**

The novel finding of our study is that aging is associated with changes in the biomechanical properties of the PCA and PAs. The effects of aging on the biomechanical properties of the posterior cerebral circulation and smaller cerebral arterioles have not been widely characterized (29). The PCA was utilized as a model of a large pial artery. The PCA is important for regulating the blood flow to the posterior cerebral circulation. The PAs serve as a bottleneck for perfusion of the neocortex (41). PAs also play an important role in determining the outcome of ischemia; however, these arterioles have not been well characterized (9, 21, 43) and the effects of aging have not been assessed. In the PCAs, aging was associated with an increase in the outer and lumen diameter and a decrease in wall-to-lumen ratio. Aging was also associated with increased wall stress and stiffness. However, wall strain and distensibility were decreased with age in the PCAs. In the PAs, no changes in the size of the artery were
observed but aging was associated with changes to the wall structure. The wall area, wall thickness and wall-to-lumen ratio of the PAs were increased with age while wall stress was reduced.

The increase in the lumen diameter of the PCA we observed with age could increase cerebral blood flow and cause hyperemia. This could be compensated for by increased myogenic tone. We did not measure myogenic tone in this study, but studies in aged mice treated with angiotensin II show age, combined with hypertension, causes a loss of myogenic tone and autoregulation in the MCA (52). Loss of myogenic tone in a large artery such as the MCA increases the risk of rupture of the PAs with fluctuations in blood pressure. In the PAs, the increased wall thickness without changes in wall stress we observed could be a positive adaptation to protect these arterioles from rupture and vascular damage.

One of the strengths of our study is the advanced age of the mice in the aged group. At 24 months old the mice used in this study were close to the end of their natural lifespan; therefore these mice truly model the geriatric population. The use of telemetric blood pressure recording in this study also is a strength because it is a more accurate technique to measure blood pressure than tail-cuff plethysmography. We also avoided the carotid catheterization approach used in many mouse telemetry studies because it may artificially alter blood pressure by affecting baroreflex function. Our studies show that mean arterial pressure and pulse pressure were increased with age. This is in contrast to studies using tail-cuff plethysmography which suggest that aging does not
affect (52) or reduces (19) blood pressure. However, our studies are in agreement with clinical studies showing that blood pressure increases with age (20, 40, 58, 55). Pulse pressure was markedly increased in the aged mice; this could lead to vascular cognitive impairment (17, 32, 56).

The higher day-night blood pressure ratio that we observed in older mice (Figure 2) is notable because this ratio is known to be an independent predictor of all-cause mortality and cardiovascular events in humans even after adjustment for 24 hour average blood pressure (11). A caveat to our study is that we do not know when blood pressure became elevated in the aged mice because blood pressure was only measured at the 24-month time point. Age alters the ability of cerebral arteries to adapt to hypertension. Cerebral arteries from young mice have the ability to functionally and structurally adapt to hypertension (38, 52). However, the MCAs from 24 month old mice have an impaired ability to respond to hypertension (51). Our studies show that age results in high blood pressure. Therefore, it is possible that the ability of the PCA and PAs from old mice to adapt to hypertension is impaired. It is also important to note that in our study we observed outward remodeling which is the opposite of what we would expect with hypertension (41). It should be noted that angiotensin II-induced hypertension is likely to have a more rapid onset than an aging-associated blood pressure change; with a more gradual increase in blood pressure the mechanisms of artery remodeling may be different.
Our preliminary studies of a small cohort of mice suggest that the ageing process also resulted in artery rarefaction, that is, a decrease in the vessel density in the brain. Cerebral artery rarefaction has been observed in some models of hypertension (37, 49) and aging (52). A reduction in the number of vessels in the brain could lead to chronic hypoperfusion (28). We also show that aging increases pulse pressure and this has been associated with artery rarefaction (50, 52). However, we do not know if the changes in PCA and PA structure observed are a causative factor in the artery rarefaction or if remodeling and rarefaction occur independent of each other.

We observed increased stiffness in the PCA but not in the PAs, suggesting that age associated changes in stiffness are different in small arterioles and large cerebral arteries. The increases in stiffness in the PCA could have been a result of higher mean arterial pressure or pulse pressure in the older mice rather than aging per se. In rat models of essential hypertension, the large cerebral arteries remodel first; this presumably serves to protect the smaller downstream arteries from the increased pressure (28). The small arteries remodel after a prolonged period of hypertension (28). It is possible that the same pattern of remodeling occurs with aging and that the cerebral artery remodeling we observed was a consequence of both aging and increased blood pressure. Further studies will be required to determine if cerebral artery stiffness in the aged mice is caused by aging itself or by increased blood pressure. Increased arterial stiffness is a hallmark of artery dysfunction and an independent predictor of cardiovascular disease (16). In peripheral arteries, aging has been associated with changes in the composition and organization of the arterial wall that
increase artery stiffness(13). We observed an increase in stiffness of the PCA without
an increase in wall thickness suggesting that increased stiffness is the result of changes
in extracellular matrix composition.

Collagen and elastin are important components of the extracellular matrix (14), and they
play key roles in maintaining the strength and elasticity of the arterial wall. With normal
aging, collagen and elastin expression is differentially regulated (13) such that the
increased stiffness in large arteries is associated with increased collagen and reduced
elastin deposition (30, 53). The increase in collagen deposition could alter the
mechanical properties of the artery wall resulting in stiffening. Mandala et al. showed
that in adult normotensive rats the amount of elastin in the PCA was reduced compared
to young rats but no changes in collagen were observed (29). Our findings suggest that
collagen deposition is increased with aging in the penetrating arterioles. The
discrepancy between these studies can be attributed to the age and strain of the
animals; Mandala et al. studied 11-12 month old Sprague Dawley rats, while we studied
mice that better represent the geriatric population (22-24 month old). The elastin
fragmentation that occurs with increased age is associated with increased expression of
the matrix metalloproteases (MMP) (18, 53, 57). MMP-2 and -9 in particular have been
associated with elastin calcification (2, 3). The ageing process also increases the
amount of calcium and phosphate in the wall (63, 64). This is associated with the
calcification of the elastic fibers and could be contribute to artery wall stiffness. We
found that the amount of calcium in the wall and the number of arteries with calcium
deposits might be increased with ageing but our data did not reach statistical
significance. We recognize that these studies were underpowered (n=3) but the number of aged mice was limited, therefore further studies will be required.

Liu et al. showed that aged mice have smaller infarct size after an ischemic stroke than young mice (28). Interestingly, however, the functional impairments in old mice post-stroke were much worse. Post-stroke the cerebral arteries are maximally dilated such that flow is proportional to the lumen diameter. Our studies showed that advancing age is associated with increased lumen and artery cross-sectional area of the PCA. This could be the cause of the smaller infarct observed in aged rats. The magnitude of the increase between the outer and lumen diameter was different. The change with aging was greater for the outer diameter of the PCA and this is likely the cause of the increase in artery stiffness. Wall thickness of the PCA was not changed with age but it was increased in the PAs. This difference implies that missing protection from high intralumenal pressure in pial arteries is associated with increased wall thickness of the downstream arterioles. In the PAs, wall thickening with age is probably a result of smooth muscle cell hypertrophy (30). Our results are consistent with previous findings showing that in human conduit arteries such as the aorta, iliac arteries and carotid arteries, aging is associated with increased lumen area and wall thickening (53). Our studies also show that aged mice have higher wall cross-sectional area in the PCA and PAs than do young mice. In contrast, in aged Fischer rats the wall cross-sectional area of the pial arteries was less than in young animals (19). Aging also altered the mechanical properties of the PCA resulting in a less distensible artery and decreased wall strain. This is consistent with the work of Hadju et al, in the pial arteries of 24 month
old Fischer rats (19). However, in another study the distensibility of the MCA from 24-month-old mice was not changed (50). The reduced distensibility we observed may be also associated with alterations of the artery stiffness.

A limitation to our study is that we did not study the development of spontaneous myogenic tone or endothelial dysfunction with age. However, it is known that aging is associated with endothelial dysfunction in the basilar artery through a reactive oxygen species dependent mechanism (34, 47). Aging also impairs the ability of the MCA to generate tone in response to static (52) and pulsatile pressure (50). Another limitation of our studies is that we did not assess the mechanism of artery remodeling; this is a topic for future study. Possible mechanisms involve changes in the arrangement of the smooth muscle cells, increases in the expression of MMPs such as MMP-2 and -9, and elastin fragmentation. We did show that the cerebral arteries of aged mice have increased collagen that could have an important role in the changes in the artery wall observed.

In summary, aging is associated with structural changes that increase the wall stiffness of the PCA and wall stress and wall thickness of the PAs; combined, these changes could result in a dysregulation of cerebral blood flow that would increase the risk of stroke and dementia. The vasculature is a potential therapeutic target for stroke and potential neuroprotective or neurorestorative therapies need a functioning vasculature to deliver the drug to the site of injury. Therefore, it is important to fully understand the mechanisms of age associated cerebral artery remodeling to improve cerebrovascular
health. For practical reasons all of our studies were conducted in male mice, therefore, further studies should be conducted in female mice to evaluate sex differences. In future studies we should assess if aging impairs vascular tone of the cerebral arteries and if the age-associated changes in cerebral artery structure are caused by the increased blood pressure or aging itself.

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Disclosures
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References


42. Pires PW, Girgla SS, McClain JL, Kaminski NE, van Rooijen N, and Dorrance AM. Improvement in middle cerebral artery structure and endothelial function
in stroke-prone spontaneously hypertensive rats after macrophage depletion. 


Yu SY, and Blumenthal HT. The calcification of elastic fibers. II. Ultramicroscopic characteristics. *J Gerontol* 18: 127-134, 1963.

**Figure Legends**

**Figure 1.** Aging increases blood pressure with no change in heart rate. Data are mean ± SEM (n = 4 for Young and n = 3 for Old) 24 hr averages of blood pressure or heart rate, as indicated, measured by telemetry are shown. Two-way ANOVA indicated significant effects of age for all blood pressures (p<0.05), but not for heart rate or activity (p > 0.05). Blood pressure data recording started on Day 3 after telemeter implantation.

**Figure 2.** Night time blood pressures are elevated in aged mice. Data are mean ± SEM (n = 4 for Young and n = 3 for Old) 1hr averages of mean arterial pressure (MAP) for the last 8 days of the data shown in Figure 1 showing substantially elevated night-time blood pressures in the aged mice. The 0 hr time point represents the first midnight of the time period shown. Two-way ANOVA indicated significant effects of time and age, with a significant interaction term (p < 0.05 for each).

**Figure 3.** Aging results in posterior cerebral artery remodeling. Outer diameter (A), lumen diameter (B), and lumen (C) area were increased with age. Wall cross-sectional area and thickness were not changed (D, E). Aging did increased wall-to-lumen ratio (F). Data are presented as mean ± SEM, ***p<0.001, **p<0.01, *p<0.05, two-way ANOVA followed by Bonferroni for post-hoc comparisons.
Figure 4. Posterior cerebral artery mechanical properties were changed with aging. Wall stress was increased (A) with age. Aging also decreased strain in the PCA (B). The PCA was less distensible with age (C). Wall stress vs strain was reduced in the older mice (D). Data are presented as mean ± SEM, ***p<0.001, **p<0.01, *p<0.05, two-way ANOVA followed by Bonferroni for post-hoc comparisons. Data from Figure 3 was used to calculate the mechanical properties of the PCA.

Figure 5. Aging increases wall thickness in the penetrating arterioles. Outer diameter (A), lumen (B) diameter, and lumen area (C) of the penetrating arterioles were not significantly changed with aging. Wall cross-sectional area (D), wall thickness (E) and wall-to-lumen (F) were increased with age. Data are presented as mean ± SEM. ***p<0.001, *p<0.05, two-way ANOVA.

Figure 6. Aging resulted in changes to the mechanical properties of the penetrating arterioles. At 120mmHg, wall stress was increased with age (A). Wall strain (B), distensibility (C), stress-strain (D) were unchanged Data are presented as mean ± SEM, *p<0.05, two-way ANOVA followed by Bonferroni for post-hoc comparisons. Data from Figure 5 was used to calculate the mechanical properties of the PCA.

Figure 7. Aging increases vascular stiffness in the posterior cerebral artery and vascular stiffness is different depending the type of artery. Wall stiffness is increased with aging in the (A) posterior cerebral artery but not in the (B) penetrating arterioles. An increased β-coefficient represented increased wall stiffness. In young mice vascular
stiffness in increased in the small arteries compared to large arteries (C). This was not the case in old mice (D). Data are presented as mean ± SEM. *p<0.05, ***p<0.0001 Student’s t-test. Data from Figure 4 and 7 was used to calculate the mechanical properties of the PCA.

**Figure 8.** Aging may increase calcium content in the wall of cerebral arteries. In a small cohort of mice, arteries with calcium deposits were counted in young and old mice. The amount of vessel with calcium deposits or the increase in the percentage of calcification (data not shown) was not significantly different between both groups (A). Representative images at 40x magnification are shown (B: positive control, C: young mouse, D: old mouse). The arrows indicate the arteries. Data are presented as mean ± SEM. p=0.3 by Student’s t-test; n=3.

**Figure 9.** Aging increases the percent of collagen deposition in cerebral arteries. In a small cohort of mice, the percentage of collagen deposits in the wall of young and old mice were quantified. Aging resulted in a significant increase in collagen deposition (A). Representative images at 40X magnification are shown (B: positive control, C: young mouse, D: old mouse). The arrows indicate the artery. An artery with increase collagen content will be stained purple. Data are presented as mean ± SEM. ****p<0.001 by Student’s t-test; n=3.
Figure 10. Aging decreases artery density. In a small cohort of mice, the amount of arteries and capillary number was quantified using Isolectin-IB4. In each animal, two images were acquired in the neocortex, one per hemisphere. Representative images are shown above. B: control, C: young, D: old. Data are presented as mean ± SEM. *p<0.05, Student’s t-test; n=3.
Young (n=8)  
Old (n=8)

Intraluminal Pressure (mmHg)

Wall Stress (dynes/cm²)

Distensibility (% increase from baseline)

Strain

Stress
A

Number of positive arteries/area

Young (n=3)  Old (n=3)

B

C

D

50 μm

50 μm

50 μm