

# Webinar Q&A Report:

## Vascular Contributions to Dementia

- 1. Does hyperglycemia, which is also associated with cardiovascular disease (CVD) and artery stiffness, lead to high glucose in the vasculature of the brain? If so, how does it negatively impact the neuronal functions that can contribute to Alzheimer's Disease (AD) development?**

Diabetes is a major risk factor for Alzheimer's disease, and there could be many reasons for the connection between these two diseases. As mentioned, hyperglycemia is associated with increased large artery stiffness. In particular, hyperglycemia increases advanced glycation end-products (AGEs) that cross-link collagen in the wall of arteries leading to increased stiffness. At the same time, hyperglycemia and AGEs also have direct negative impacts on the endothelial cells, neurons, and other brain cells leading to increased oxidative stress and inflammatory signaling.

- 2. You mentioned that amyloid-beta (ABeta) accumulation causes endothelial dysfunction, but the opposite also seems plausible. Can you expand on which comes first?**

We don't know which one comes first but knowing this would support better design of preventions/treatments. Amyloid-beta causes cerebral endothelial cell dysfunction, and cerebrovascular dysfunction leads to increased amyloid-beta accumulation (due to limited blood flow, increased blood barrier permeability, poor removal of amyloid-beta from the brain, etc.). Thus, these two (amyloid-beta and endothelial dysfunction) create a vicious cycle. Philosophically, most treatments are designed to target one or the other, and perhaps targeting both at the same time would have the most benefit.

- 3. What are your thoughts on the hypercapnia protocol as a method to assess cerebral reactivity?**

Hypercapnia is often used in human studies to determine cerebrovascular reactivity. There is some evidence that the actions of hypercapnia are via stimulated nitric oxide production, and thus, this is relevant to our ex vivo artery studies. An advantage of this technique is a catheter is not required (e.g., for drug injections), making this technique more reasonable for long-term repeated measures studies. Also, because this technique uses MRI, we can measure perfusion in deep brain regions that are not accessible with Doppler or microscopy. However, there are limitations of this protocol. In particular, not all brain regions are equally reactive to hypercapnia. In addition, the reaction to hypercapnia is not as physiologically relevant to dementia as the reaction to reaction to neuron stimulation (i.e., measures of neurovascular coupling).

#### **4. Does arterial stiffness or ABeta accumulation develop first?**

Data from large studies in humans suggests that the appearance of arterial stiffening (about 50 years of age) occurs earlier than the onset of amyloid-beta accumulation (about 60-70 years of age). However, these data are limited by the sensitivity of the measurement techniques, and it is possible that amyloid-beta is accumulating in the brain before it can be detected. We believe that increased arterial stiffness leads to cerebrovascular dysfunction and increased neuroinflammation, both of which trigger increased amyloid-beta production. At the same time, it is possible that amyloid-beta accumulation, particularly within the vasculature (e.g., cerebral amyloid angiopathy), increases the susceptibility to detrimental impact of stiffer large arteries. Likely, it is a combination/interaction of the vascular dysfunction and amyloid-beta (and other factors) that are cause late-onset Alzheimer's disease, a multi-factorial disease.

#### **5. Do you see ABeta accumulation around arteries in your AD mice?**

With the models we used thus far, we do not find accumulation of amyloid-beta around the arteries. However, our studies were performed with the 3xTg-AD mouse, a model not known for vascular amyloid-beta accumulation. Perhaps we would find more cerebrovascular amyloid if we used a model with mutations more commonly associated with cerebral amyloid angiopathy (e.g., APP –Dutch) or a model where mutant proteins (APP or otherwise) are expressed in vascular cells.

#### **6. What is the sex of the mice used in your studies, and what is the role of sex in vascular aging?**

Some of these studies were performed only in male mice (e.g., our original work with the Eln+/- mouse), while some use both males and females (e.g., the Eln+/- mouse crossed with the 3xTg-AD). For those studies performed only in male mice, we are working to repeat the studies in females. We do not have enough data yet to identify sex differences in the outcomes.

In a recent study from my lab, we found that female and male mice have a decline in cerebral artery endothelial function with aging. In that study, we related the endothelial dysfunction to frailty index, a marker of "biological age." We found that cerebral artery endothelial dysfunction was related to a higher frailty index among old females, and this was independent of chronological age. However, there was no relation between cerebral artery endothelial function and frailty among old male mice. This suggests that cerebral artery dysfunction is more related to declines in general health among females compared with males. Here is a link to read more about this study: <https://pubmed.ncbi.nlm.nih.gov/34664649/>

We also have not examined the effects of estrogen or menopause/ovariectomy with the models of large artery stiffness or high pulse pressure. It is known that large artery stiffness increases post-menopause. There is also evidence that the age-related increase in large artery stiffness occur more rapidly in human females compared with males. The reason for this more rapid onset of arterial stiffness in females may be that estrogen is protective against stiffening in the pre-menopausal state, and the dramatic decline in estrogen with menopause leads to a rapid increase in large artery stiffness. Potentially, this rapid increase in arterial stiffness is more detrimental to the cerebral vasculature due to less time for adaptation compared with the slower increase in

stiffness in males. However, this hypothesis remains to be experimentally tested. We recently wrote a mini review on this topic: <https://pubmed.ncbi.nlm.nih.gov/35072153/>

#### **7. How do you assess the pulse wave velocity in mice?**

We assess pulse wave velocity in the aorta of mice. We start by recording the arrival time of the pulse at the aortic arch and the abdominal aorta. We collect the pulse wave forms at these two locations simultaneously using Doppler flow probes. We then calculate pulse wave velocity as the distance between the probes, divided by the difference in arrive time between the probes. A limitation to this measurement is that it must be performed on mice while under anesthesia. This article further describes the methods for measuring arterial stiffness in rodents:

<https://pubmed.ncbi.nlm.nih.gov/32268787/>

#### **8. Are you interested in studying late vs. early onset AD mice models with your perfusion models?**

We believe that arterial dysfunction is more likely to have a role in late-onset Alzheimer's disease. Admittedly, our first studies were with the 3xTg-AD mouse, a model that uses mutations associated with early-onset Alzheimer's disease. We chose this model initially because it allowed us to study the interaction of aberrant amyloid-beta and tau pathology with greater arterial stiffness. In the future, we are interested in increasing the translatability of these findings by using a model more similar to late-onset Alzheimer's disease, such as the humanized APP mouse.

#### **9. Can you specify the age of the "old" and "young" mice?**

For C57BL6 mice, we classify "old" as 24-28 months and "young" as 4-8 months of age.

#### **10. What is the mean lifespan of heterozygous mice?**

As far as I know, longevity has not been studied in the elastin heterozygote mouse model. For the data I presented, we studied these mice at a young age (4-8 months). Our goal was to model age-related increases in arterial stiffness in a young mouse, thus isolating the effects of arterial stiffness.

#### **11. The relationship of microinfarcts with the onset of AD has been described. Is there any study that relates a high pulse pressure in the cerebral arteries with the risk of suffering microinfarcts?**

It seems likely that increased large artery stiffness and higher pulse pressure should be associated with more microinfarcts, but we have yet to examine this in our models. Given the limitations with measuring microinfarcts by imaging in humans, there is a lack of studies about these associations in humans as well. However, there are a number of studies that find a relation of greater large artery stiffness or higher pulse pressure with other indicators of cerebral small vessel disease, such as white matter hyperintensities. Here are a few examples:

<https://pubmed.ncbi.nlm.nih.gov/29549223/>

<https://pubmed.ncbi.nlm.nih.gov/23172923/>

<https://pubmed.ncbi.nlm.nih.gov/22075523/>

<https://pubmed.ncbi.nlm.nih.gov/28389742/>

**12. Down to what level of the cerebrovascular tree do you expect dysfunction of the endothelium?**

Thus far, in our model of increased large artery stiffness (the Eln+/- mouse) we find endothelial dysfunction in cerebral arteries. However, I would expect that increased large artery stiffness would have a greater impact on the more fragile microvasculature compared with the cerebral arteries. We are performing experiments now to examine the effects on the microvasculature.

**13. Are other extracellular matrix components increased in the large arteries to contribute to stiffness?**

With aging, the major extracellular matrix changes to large arteries include increased collagen, increased collagen cross-linking, and fragmentation of elastin (for more information see <https://pubmed.ncbi.nlm.nih.gov/34779281/>). We use the elastin heterozygote mouse because it is a strong model of greater large artery stiffness with minimal other effects; however, we acknowledge that lower elastin content is not a primary cause of age-related arterial stiffening.

**14. Does this experimental context remove these vessels autoregulatory-myogenic responses?**

During our ex vivo pulse studies, the myogenic response of the arteries is still present. While we did not measure this myogenic response in these studies, previous work by Springo et. al. indicates that cerebral arteries from old mice have less myogenic tone after exposure to high pulse pressure compared with young mice (see <https://pubmed.ncbi.nlm.nih.gov/25605292/>). An impaired myogenic response in the old cerebral arteries will all the high pulse pressure to further penetrate the microvasculature, thus exacerbating the negative effects of high pulse pressure on the microvasculature.

**15. Can you expand on how your lab is looking at reversing these vascular events?**

We are currently testing interventions to prevent or reverse age-related increases in arterial stiffness. We recently studied pyridoxamine, a form of vitamin B6 that prevents the formation of advanced glycation end-products. We found long-term pyridoxamine treatment prevented age-related increases in large artery stiffness, preserved cerebral artery endothelial function, and had modest benefits for cognitive function (manuscript currently under review). Other potential pharmaceuticals include the collagen cross-link breaker alagebrium. Some of the strongest benefits for preventing age-related increases in arterial stiffness come from lifestyle interventions, such as aerobic exercise and dietary factors (see <https://pubmed.ncbi.nlm.nih.gov/30212305/>).

**16. Do you think that vascular reactivity is a better measure of vascular dysfunction than PWV as an indicator of the likelihood of cognitive dysfunction?**

In our work, we are more focused on understanding the mechanisms and initiating events between these two. Studies from humans indicate that markers of both arterial stiffness and cerebrovascular function are related to cognitive impairment. While there are studies that suggest

that arterial stiffness is a stronger marker for cognitive impairment, other studies suggest that arterial stiffness and endothelial function are equally strong.

**17. What type of inflammation occurs in the brain that's associated with increased artery stiffness?**

While studies have found T-cells present in the brain with aging and Alzheimer's disease, we have not measured T-cell content in our models of large artery stiffness. We do find increased inflammatory signaling in the cerebral arteries and brain, in particular elevated interleukin-1 beta. In addition, we find more activated microglia in our mouse model of large artery stiffness.

**18. Broadly, how is cognitive impairment being defined within the various models you have mentioned? Do the cognitive outcomes depend on the specific dysfunction?**

The cognitive impairments most related to arterial stiffness (and pulse pressure) are impairments in memory and learning. Greater large artery stiffness has been associated with impaired executive function and memory in human studies (a recent meta-analysis: <https://pubmed.ncbi.nlm.nih.gov/32106748/>) and memory in mice (<https://pubmed.ncbi.nlm.nih.gov/31057061/>).

**19. Eln+/- mice should have systemic endothelial cell (EC) dysfunction. What happens in their heart and coronary arteries?**

We do not find that Eln+/- mice have systemic endothelial cell dysfunction compared with Eln+/+ mice. For example, we find that there is no endothelial dysfunction in skeletal muscle feed arteries of Eln+/- mice (<https://pubmed.ncbi.nlm.nih.gov/25627876/>). Others have found no dysfunction in the aorta or carotid arteries of Eln+/- mice (<https://pubmed.ncbi.nlm.nih.gov/14597767/>). The Eln+/- mouse was originally created as a model of supravalvular aortic stenosis, and thus, a limitation to these studies is that this model does have impaired heart valve function (<https://pubmed.ncbi.nlm.nih.gov/9819363/>).



Copyright 2022 InsideScientific Corporation. All rights Reserved.