WEBINAR: Aging and the Cardiovascular System - An *in vivo* and *in vitro* approach to the pre-clinical assessment of arterial stiffness

Questions and answers from the webinar held on June 10th, 2020.

This document includes questions we received and answered during the webinar, as well as those that we did not have time to address while online.

Q Is the procedure for Pulse Wave Velocity (PWV) an established procedure in the literature? Do you have a video link for the procedure?

A Please follow this link to a document entitled "Performing Pulse Wave Velocity Measurements using the Doppler Flow Velocity System"

You can see a short video here that describes this procedure in more detail and shows the same video clip that was presented during the webinar of how PWV is collected on an anesthetized mouse in the lab.

Q For Pulse Wave Velocity, can you measure flow from two different regions other than the aorta region?

A You can absolutely measure PWV from vessels other than the aorta. Please reach out to our application specialists to discuss your specific vessels of interest and we can direct you to the system configuration that will meet your needs.

Q Which would you say is a better or more efficient method of measuring arterial stiffness: Doppler or pressure myography?

A Both can be efficient; it depends what the goal of your study is. If you are looking to do longitudinal aging studies *in vivo*, then PWV is best. If you are looking at *in vitro* changes due to pharmacological intervention or manually changing the pressure to see vessel responses, Pressure Arteriography might be better suited to your study.

Q Why do some labs advise measuring blood pressure when measuring PWV?

A Pressure is a valuable tool and works nicely for PWV, but it often renders the procedure terminal and thus makes it difficult to arrange for longitudinal studies. Since Pulse Wave velocity is technically a pressure wave rolling through the artery, it is very logical you would want to measure the pressure changes, however with increased pressure comes increased velocity, and thus measuring the onset of velocity increase in the segment of the artery is equally valuable and precise. The only difference is that one can measure the velocity non-invasively, and thus making it more suited for aging and longitudinal studies.

Q How sensitive is the Doppler system and how does it correct other disturbances from the body?

A The Doppler Flow Velocity System is quite sensitive and can measure flows as low as 5cm/s. It usually does not need to correct for body disturbances as the probe is placed on the skin surface and any movement of the body also moves the probe tip usually the same amount.

Q Is it possible to use continuous invasive blood pressure waveforms to calculate PWV?

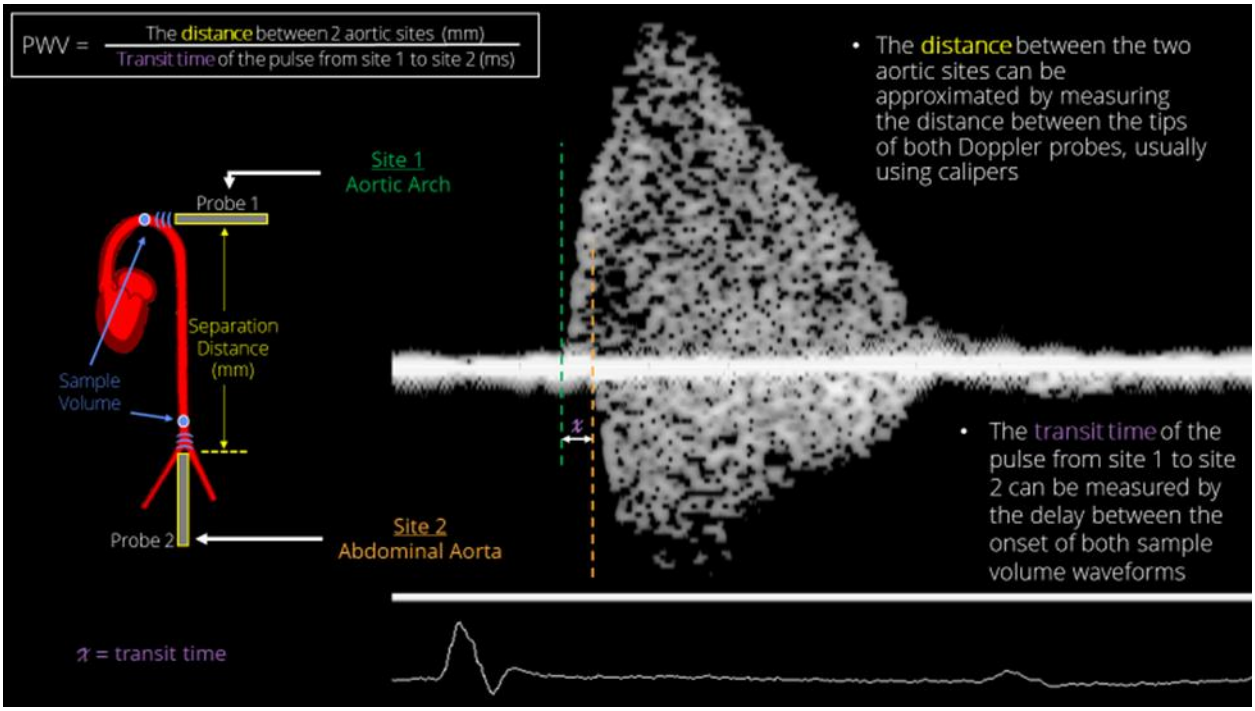
A Yes, and this is a popular method when two invasive pressure catheters are inserted into the vessel sites of interest during surgery. This procedure is usually terminal though, and results in sacrifice of the animal at the end point of the surgery.

Q Is it possible to measure PWV using just one ultrasound probe, by measuring the delay between the ECG QRS wave and the upslope of the echo pulse at each probe?

A Yes, this is the way it was done previously, but the risk of error increases significantly because the heart rate of an animal under ISO is so variable you cannot guarantee that the HR did not change between signals. Now with simultaneous Doppler with the 2-channel DFVS you can avoid that error all together because you can collect both signals at the same time and thus on the same heartbeat/rate.

Q You mentioned PWV measures the pressure propagation velocity, but the doppler is measuring a velocity. Is this measured velocity different than the stroke volume penetration velocity?

A The doppler is measuring a velocity yes, but that velocity is not the one being reported. The velocity being reported as PWV is the distance between the sample volumes (estimated with calipers) divided by the transit time (the timing delay between onset of the Doppler velocity waveforms). Distance over time gives a velocity, which is much higher than any velocity you will see measured by the doppler directly.



Q How precise can you determine the vessel length with this approach and what advantages does it have over invasive methods?

A The precision of vessel length depends on the consistency of the user. If the user is careful with caliper tip placement, the distance can be approximated quite confidently. The advantage over invasive pressure is that the animal does not need to be sacrificed at the end of the procedure so they can become their own control as disease or age progresses. This makes the progression of disease easier to quantify. It is hard to measure age related affects when the animals are not able to age due to invasive procedures and termination.

Q I noticed that wire myography was not discussed in the webinar...is it still a good method to assess vascular reactivity?

A Wire myography is an acceptable method for assessing vascular reactivity. Its advantages include the ability to carry out high throughput experiments, study large vessels such as the aorta (too opaque for pressurized arteriography), and an overall easier experimental setup. That said, pressurized arteriography provides more detailed information on the true physiologic mechanics of vessel constriction and dilation (closer to the *in vivo* condition), as opposed to the more simplistic isometric force measurement of wire myography.

Q Which would you say is a better or more efficient method of measuring arterial stiffness: Doppler or pressure myography?

A Strictly speaking, the Doppler Flow Velocity System provides the more direct measure of arterial stiffness. Pressurized arteriography can further elucidate what may be responsible for these *in vivo* changes in arterial stiffness (impaired endothelial function, for example). It depends on the experimental model and research objectives. Ideally, both systems can be used to provide a more complete understanding of vascular function.

Q Is the perfusion fluid warmed and oxygenated? Is the superfusion buffer oxygenated?

A Both perfusion and superfusion buffers should be warmed and oxygenated to physiologic norms. The single vessel chambers also have a clear cover which is used to help maintain chamber environment for longer experiments (several hours), or for researchers who want to control the gas mixture, such as in hypoxia studies, for example.

Q Does time of the day (circadian) affect myogenic tone?

A It is well known that circadian rhythms do affect the cardiovascular system. The extent to which does depend on the species model and other factors. Best laboratory practice would be to euthanize the animals at the same time each day. There are a few cases we've heard about where research results were impacted because the researchers were euthanizing animals and collecting tissues at all different times of the day. Conversely, pressure arteriography would be an excellent investigatory tool to quantify circadian rhythm impact on vascular reactivity.

Q If the perfusion is not pulsatile, can this cause the vascular diameter and reactivity to differ from the *in vivo* behavior?

A Yes, technically the lack of the pulsatile environment is a departure from the *in vivo* state. That said, resistance vessels are exposed to a lower pulsatile blood pressure and flow environment given they are so far downstream from the heart and aorta. Like any *in vitro* model, the ability to study a tissue or vessel in isolation is advantageous if results are properly interpreted in their context. Not discussed in this webinar, pressurized arteriography can also be configured to control for pressure and flow, where both vessel ends are open-cannulated so that the buffer flows through the vessels giving the researcher the ability to measure flow-mediated vascular responses, a little closer to the *in vivo* condition.

Q How much time is required to perform a pressurized arteriography measurement? How much time is spent at each pressure step? Does vessel viability and reactivity decay over time?

A The amount time required to perform pressurized arteriography depends on the skill level of the investigator (which can be improved with practice) and the protocol itself. Typical durations for pressurized arteriography experiments are between 1 to 4 hrs. Vessels can last up to 6-8 hours, beyond that, loss of vascular reactivity and function will be observed. Vessels can be harvested and stored overnight, but best practice is to use them soon after harvesting on the same day.

For an excellent introduction into the setup of a typical pressurized arteriography experiment, we recommend the webinar by Prof. Scott Earley, PhD, University of Nevada School of Medicine, hosted by Living Systems Instrumentation:
https://www.livingsys.com/learning-resources/webinar-resources/getting-started-with-in-vitro-blood-vessel-research-february-24-2015/