

## In vivo ultrasound protocol

As mentioned in the initial application, we proposed to use high frequency ultrasound for the sequential detection of luminal diameter of mice during saline and AngII-infusion. At the time of the previous submission, we had been advised by Dr. Wang (Berlex Bioscience) about the ability of the machine from Visualsonics to perform these measurements. Recently, Dr. Wang's group has published on their ability to detect aortic dimensions using this machine.<sup>1</sup> Since the previous submission, we purchased this ultrasound machine with a 704 scanhead that has a frequency of 40 MHz, a focal length of 6 mm, and a resolution of 30  $\mu$ m. We have three operators that have been trained by Visualsonics in the use of the in vivo ultrasound and have been performing studies to determine its utility to noninvasively detect lumen diameter during AngII-induced AAA formation. We used the suprarenal region of the abdominal aorta, immediately above the right renal artery, as a reference point to obtain these measurements. Three operators performed ultrasound measurements of lumen diameter on saline (n = 3) and AngII-infused (n = 10) mice at 7 different time points during the course of a 28 day infusion (Fig 1). The intra- and inter-operator error for measurements of lumen diameter by ultrasound by these three operators was below 10%, even at this fairly early stage of our experience. Our operators can acquire the aortic dimension of a specific region within 1 minute of initiating the scan, making it feasible to perform this measurement on many mice each day. As seen in Fig 1, we can easily discern the large luminal expansion that develops during the infusion of AngII, which begins to increase within 0 to 5 days from the start of infusion. In vivo ultrasound imaging of the abdominal aorta was performed on day 28 in mice from each group; sample images are provided in Figure 2. The lumen diameter was 2-fold greater in the abdominal aorta from an AngII-infused apoE<sup>-/-</sup> mouse (Figure 2B) compared to saline (Figure 2A). The total lumen circumference (lumen perimeter) for each vessel was also determined. Longitudinal images were also obtained from these mice (bottom panels of Figure 2). Figure 3 illustrates the vessels in situ from these same mice (top). The external diameter of the excised aorta from each of these mice is illustrated in the bottom panels of Figure 3. Interestingly, comparison of the lumen (by ultrasound) to external diameter of the excised abdominal aorta from a saline-infused mouse demonstrates that the lumen represents approximately 94% of the external diameter measurement. In contrast, the lumen represents approximately 66% of the external diameter measurement in the aorta from an AngII-infused mouse. This difference is most likely attributed to the marked vascular remodeling in a formed AAA. Importantly, non-invasive ultrasound measurements of lumen diameter accurately reflected the treatment effect of AngII that was observed using the conventional measurement of external diameter on excised tissue. Thus, in addition to terminal measurements of external aortic diameter on excised tissue, non-invasive measurement of lumen diameter by in vivo ultrasound will be used in Core C to chronically evaluate lumen dimensions during the evolution of AAA formation. In ongoing studies to determine the rate of luminal expansion during AngII infusion, ultrasound imaging is being performed every other day during the initial 10 days. This is the interval of greatest change in luminal dimension.<sup>16</sup> The mice tolerate this procedure with no apparent detriment to their health.