EFFECTS OF CHRONIC HYPERTENSION ON CARDIAC MYOCYTE PHYSIOLOGY REVEALED THROUGH REMOTE FOCUSING MICROSCOPY.

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Summary: Images are presented showing direct measurements of sub-cellular sarcomere spacing within a living rodent heart. Systematic changes in the cell structure of chronically hypertensive rats are reported.

Background: Cardiomyocytes are the cellular powerhouses that allow the heart muscle to contract. The electrical and mechanical properties of these specialised heart cells can be characterised according to the spacing of sub-cellular sarcomere structures. Identifying systematic changes in sarcomere length between chronically hypertensive and non-hypertensive rats can help to close the gap in understanding between microscopic and macroscopic models of hypertension.

Remote Focussing microscopy: Until recently it was only possible to estimate the sarcomere length of cardiomyocytes. Focal plane images of the cells resolved the sarcomere structures, but not the tilt angle between the cell and the focal plane required to calculate the true spacing from the image. Remote focussing microscopy is a technique that allows the focal point of a laser scanning microscope to move axially without distortion at kHz rates to enable the rapid imaging of objects outside the focal plane. Imaging cardiomyocytes quickly removes the effects of movement distortions during the beat cycle, allowing the resting sarcomere length to be resolved. Imaging planes rotated at +45 degrees and -45 degrees to the focal plane, we are able to uniquely resolve the cell orientation and recover the true sarcomere spacing information.

Results: Extracting the true distribution of sarcomere lengths from multiple sites across the heart we were able to isolate subtle, systemic changes in cell health between hearts that would otherwise be lost by the artificially broadened focal plane distribution. The results of this study include over 450 individual cell plane measurements, across 6 hypertensive and 3 non-HT rodent hearts. Systematic differences in cell physiology between hypertensive and non-hypertensive groups are reported.

Figure 1: Two-photon fluorescence images of cardiomyocytes taken in focal plane (left) together with out-of-plane cell images (above)