

Structural Basis of Atrial Arrhythmogenesis in Metabolic Syndrome

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Background: Metabolic syndrome (MetS) is a fast-rising global epidemic defined as a cluster of metabolic derangements, including insulin resistance, abdominal obesity, dyslipidemia, and increased blood pressure. It is a significant risk factor for atrial fibrillation (AF), the most common cardiac arrhythmia. Individual components of MetS are intimately associated with AF, but the exact mechanism underlying the increased susceptibility to AF in MetS patients remains unclear.

Objective: This study aims to identify the key structural substrates of AF in MetS.

Methods: Adult New Zealand white rabbits were fed *ad libitum* with a high-fat/high-sucrose diet to induce MetS for 28 weeks (N=3), alongside age-matched controls (N=3) and euthanized in Valencia. The rabbit atria were then isolated and processed using a novel CUBIC clearing protocol in Auckland. Next, the atrial tissue was incubated in wheat germ agglutinin (WGA) to label cell membranes and collagen. A confocal microscope was used to image the tissue with an isotropic resolution of $\sim 0.2\mu\text{m}^3$. The collagen and cell membranes were segmented using a robust machine learning architecture, *U-net*. Collagen quantification was done by calculating the total pixels identified in each segmented image. Cell measurements were taken using ImageJ.

Results: The optimal clearing time for the rabbit atrial tissue was ~ 1 hour and the incubation time in WGA was 20-25 mins for $20\mu\text{m}$ sections. The atria of the MetS rabbits were dilated and there was patchy fibrosis in the left and right atria. Furthermore, we observed increased presence of collagen in MetS atrial tissue, which was interwoven between the cardiomyocytes. In the MetS tissue, the cardiomyocytes were hypertrophied ($14.447\pm 3.614\mu\text{m}$; n=25) in comparison to control ($9.467\pm 1.769\mu\text{m}$; n=25).

Conclusion: Compared to the control, atrial fibrosis, cell hypertrophy, and atrial dilatation were identified in the MetS rabbits. This structural remodeling may be the arrhythmogenic phenotype which leads to increased AF susceptibility.