SUBSTRATE FOR ATRIAL FIBRILLATION IN CARDIOMYOPATHIES

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To my dad Kah Ding

my wife Phoebe

and my children Justus & Hayley

In loving memory of my mum Suok King

Table of Contents

Abstract

Atrial Fibrillation is the most common heart rhythm disorder. However, our understanding of the underlying patho-physiological mechanisms of AF remains limited. Both hypertension and heart failure are known to play an important role as risk factors for AF. With the increase in the incidence and prevalence of both these conditions and the predicted atrial fibrillation epidemic, their underlying mechanistic associations require careful attention. This thesis focused on the evaluation of atrial remodeling in large animal models of these common substrates.

Chapter 2 presents the detailed anatomical, histological and functional characterization of the cardiac changes in the ovine “one-kidney, one-clip” model of hypertension using state of the art cardiac magnetic resonance imaging. Chapter 3 presents the significant atrial electrical, structural and functional remodeling evident with short duration (mean of 7 weeks) of hypertension. Pivotal changes were seen in increased atrial interstitial fibrosis and the resultant conduction abnormalities. This highlighted the importance of early and aggressive therapy of hypertension which may prevent the development of an arrhythmogenic atrial substrate.

Chapter 4 examines the time course of atrial remodeling during the development of hypertension over a period of 15 weeks. Anatomical and

functional remodeling started early while structural changes in increased fibrosis occurred later in the remodeling process. The early changes were associated with increased atrial fibrillation inducibility while the late changes were associated with more prolonged induced atrial fibrillation episodes. This understanding of the time course of remodeling provided important insights, whereby a narrow window of opportunity exists for preventing more permanent structural changes that can sustain atrial fibrillation. This work also implicates the need to maintain good blood pressure levels in atrial fibrillation patients. In particular, recent evidence has shown that pre-hypertension is associated with increased incidence of atrial fibrillation.

To date, experimental studies on atrial remodeling in heart failure had utilized one single animal model of rapid ventricular tachypacing induced heart failure. This model may not be representative of all types of cardiomyopathy in the heart failure syndrome since different underlying causes of heart failure have been shown to portend different prognostic value. Chapter 5 further evaluates atrial remodeling in heart failure using a recently characterized ovine model of non-reversible doxorubicin-induced non-ischemic cardiomyopathy. The main feature of atrial remodeling lies in the structural changes of atrial interstitial fibrosis with increased conduction heterogeneity which resulted in longer induced atrial fibrillation episodes. These findings suggest a consistent substrate

for atrial fibrillation in different heart failure models indicating ‘remodeling of the same sort’.

Chapter 6 presents the atrial effects of omega-3 fatty acids treatment in ovine heart failure. Omega-3 fatty acids prevented atrial enlargement, reduced atrial fibrosis and the related conduction abnormalities resulting in shorter atrial fibrillation episodes. Clinically, omega-3 fatty acids have been shown to provide additional albeit modest improvement in outcomes of heart failure patients above current evidence-based therapies. Therefore, omega-3 fatty acids may potentially provide a relatively affordable and non-toxic option to prevent adverse atrial remodeling and reduce atrial fibrillation burden in this subgroup of patients with heart failure.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Dennis Lau and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Publications and Communications to Learned Societies

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1. Research Prize for best scientific oral presentation, Australian Chinese Medical Association (SA) 7th Annual Scientific Meeting 2008

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3. Nimmo Prize for best scientific oral presentation (full-time research category), The Royal Adelaide Hospital 2008 – Winner

4. Young Investigator Award (First Prize), 1st Asia Pacific Heart Rhythm Society Scientific Session 2008, Singapore

5. Best Poster Award (First Place), American College of Cardiology 58th Annual Scientific Session 2009, Orlando, FL, USA

6. Nimmo Prize for best scientific oral presentation (full-time research category), The Royal Adelaide Hospital 2009 – Finalist

7. Best Research Poster, The University of Adelaide, Faculty of Health Sciences Postgraduate Research Expo 2009

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Chapter One

Literature Review

1.1 Introduction

Despite the tremendous advances made in cardiovascular medicine over the last century, atrial fibrillation (AF) has emerged as an epidemic of the new millennium.[1] Current estimated prevalence of AF in unselected adult population is approximately 1%. However, this has been shown to increase with age from 0.1% in those less than 55 years old to 9% in those over 80 years old. [2] What is of concern is that the prevalence of AF will increase by an estimated 2.5 to 3 fold by the year 2050.[2, 3] This will translate to significant economic burden on health care systems worldwide given the considerable morbidity and mortality associated with this condition.[4]

1.1.1 Consequences of Atrial Fibrillation

Clinical symptoms commonly associated with AF include palpitations, fatigue, chest discomfort and dyspnea. More importantly, AF has been found to confer increased risk of cardiac failure and thromboembolic events leading to increased hospitalizations.[5, 6] Specifically, the adjusted risk for both cardiac failure and stroke increases by up to 3.5 and 5 times respectively with AF [5, 7, 8] with almost 15% of all strokes attributable to this arrhythmia.[9] More recently, AF has also been linked with increased dementia independent of stroke

history.[10, 11] In addition to the increased morbidities, data from the Framingham Heart Study showed an increase in the relative risk of death by 1.5 to 1.9 fold as a result of AF independent of other cardiovascular conditions.[12] Similar mortality burden has also been replicated from an Australian survey.[13]

1.1.2 Current Management of Atrial Fibrillation

In the absence of a curative therapy, the management of AF has been limited to stroke prevention, rate control or maintenance of sinus rhythm. The overwhelming evidence for warfarin anticoagulation in primary and secondary stroke prevention is based on multiple prospective randomized studies showing significant risk reduction of more than 80%.[14-18] In contrast, anti-platelet agent such as aspirin has comparatively lower efficacy showing risk reduction by about one-fifth only.[19] Newer strategies in stroke prevention are being studied with promising results in the form of new anticoagulation agent with Dabigatran (direct thrombin inhibitor) and percutaneous left atrial appendage occlusion.[20, 21]

Pharmacological management of AF has been limited by the non-availability of atrial-specific anti-arrhythmic agents and the significant side effects profile of current available drugs. Although recent studies have demonstrated no survival advantage with rhythm over rate control strategy in patients with AF, this may

be accounted for by the adverse profiles of current anti-arrhythmic drugs since sinus rhythm remains an important determinant of survival.[22-24] The development of new anti-AF agents has been slow with only one new drug, dronedarone, approved for use over the last decade. Yet, this does not represent a clear step forward given that the gain in safety with fewer adverse side effects is offset by lower anti-arrhythmic efficacy.[25-28]

The last decade has also witnessed the evolution of catheter AF ablation from an experimental procedure to a recommended treatment in those with symptomatic drug refractory AF.[29] Of note, the success rate of this type of procedure varies with the type of AF treated, ablation techniques employed, operator's experience, concurrent usage of anti-arrhythmic drugs and number of additional ablation procedures.[30] This worldwide survey also reported the significant risk of procedure related major complications of between 4.5 to 6%.[30, 31] The differences in ablation techniques amongst electrophysiologists also highlighted the current knowledge gap in the underlying mechanisms of this complex arrhythmia.

1.2 Mechanisms of Atrial Fibrillation

Since the initial recognition of this arrhythmia in the early 20th century as an irregular rhythm originating from the auricles,[32] several different theories have

been purported as possible underlying mechanisms. Here, the major schools of thought are outlined and discussed:

i. The multiple wavelet hypothesis
ii. Focal electrical discharges
iii. Localized re-entrant activity with fibrillatory conduction
iv. Rotors with fibrillatory conduction

1.2.1 The Multiple Wavelet Hypothesis

In the 1960s, Gordon Moe was first to hypothesize that multiple meandering independent wavelets coexist in the fibrillating atria, after being fractionated from an initial wavefront as it encountered refractory tissues.[33, 34] Temporal dispersion of refractoriness, sufficient atrial tissue mass, brevity of the refractory period and conduction slowing were factors thought to be important determinants for the maintenance of AF. Experimental support of this theory was first demonstrated by Allessie and co-workers during mapping of acetylcholine induced AF. In this canine model, they estimated a minimum of 4 to 6 wavelets were needed to maintain the arrhythmia.[35] This concept was further strengthened by experimental anti-arrhythmic studies whereby increased wavelength and decreased number of wavelets preceded the termination of AF.[36-38]

In the clinical setting, Cox et al. studied patients with paroxysmal AF undergoing surgical correction of Wolff-Parkinson-White syndrome and demonstrated that multiple wave fronts, non-uniform conduction, bidirectional block, and large macro-reentrant circuits occur during AF.[39] This electrophysiological phenomenon provided the basis for the surgical MAZE procedure, whereby compartmentalization of the atria led to interruption of the multiple wavelets capable of sustaining AF.[40] Likewise, percutaneous catheter ablation for AF was pursued based on this early surgical work. In addition, mapping studies during pacing induced human AF by Konings and co-workers, demonstrated various types of AF characterized by different numbers and dimensions of the re-entrant circuits depending on conduction slowing secondary to arcs of conduction block.[41]

1.2.2 Focal Electrical Discharges

The landmark observation that AF can be initiated by focal electrical triggers was elegantly described by Haissaguerre and co-workers following their successful attempts with catheter ablation in the 1990s.[42-44] The pulmonary veins are now recognized as the crucial source of triggers which initiate AF[44] although pre-clinical observations of AF originating from the thoracic veins had been reported since the 1970s.[45] Indeed, Scherf and co-workers had also reported in the 1940s that AF could result from rapidly firing foci in their animal

studies of aconitine induced atrial tachycardia.[46, 47] However, the mechanisms underlying spontaneous focal pulmonary vein ectopy are not yet fully understood.

The presence of specialized conduction tissues in the myocardial sleeves of the pulmonary veins may partly explain their arrhythmogenicity.[48-50] Isolated cardiomyocytes from canine and rabbit pulmonary veins have also exhibited abnormal automaticity and triggered activity as well as both delayed and early after-depolarizations following rapid atrial pacing.[51-54] The observation of tachycardia-pause initiation of rapid, short-coupled pulmonary ectopy in patients with AF lends further evidence to triggered activity as the likely mechanism.[55] Nevertheless, isolation of the pulmonary veins is now the cornerstone of most AF ablation techniques given these new understandings of the pulmonary vein physiology.[29]

Several other non-pulmonary vein foci have since been found to initiate AF. These include: superior vena cava, coronary sinus, ligament of Marshall, crista terminalis, posterior left atrial wall, interatrial septum and tricuspid/mitral valve annuli. [42, 43, 56-59] Moreover, AF can also be initiated following degeneration of other atrial tachycardia or supraventricular tachycardia, often in the environment of an underlying AF substrate. It has been shown that ablation of accessory pathway mediated tachycardia could prevent AF recurrence.[60]

1.2.3 Localized Re-entry with Fibrillatory Conduction

Areas of localized re-entry that can initiate AF have been observed in the pulmonary veins and posterior left atrium. The requirements for re-entry are listed below:[61]

i. Uni-directional conduction block;
ii. A core of in-excitable tissue around which a wavefront propagates; and
iii. The maintenance of excitable tissue ahead of the propagating wavefront ("the excitable gap").

The normal anatomic extension of myocardial tissues into the pulmonary veins had been known since the work of Nathan and Eliakin.[62] Detailed anatomic characterization of this region was not performed until recently, showing a variable extension of myocardial sleeves of between 1-3cm and a variable thickness along the pulmonary veins.[63, 64] The length of the myocardial sleeves was found to be greater in the superior pulmonary veins than their inferior counterparts, corresponding to the greater number of ectopic foci seen.[44, 65] There was also experimental evidence for the complex arrangements of myocardial fibers in the pulmonary veins showing abrupt changes in fiber direction and short fibers arranged in mixed direction, which correlated with zones of conduction delay and fractionated electrograms.[66, 67] In the clinical

setting, decremental conduction from the pulmonary vein to the atrium and ERP heterogeneity has also been observed.[68, 69] These distinctive electrophysiological properties may therefore facilitate local re-entry and lead to increased arrhythmogenesis.

The posterior left atrium has been identified to harbor the earliest of fibrillatory activity with the highest amount of disorganization, highest dominant frequency and shortest cycle length in multiple clinical and experimental studies.[70-75] Not surprisingly, in various AF ablation approaches, the inclusion of pulmonary venous isolation together with the posterior left atrium resulted in improved long-term success.[76, 77] Further evidence of the importance of the posterior left atrium was available from the work of Todd and co-workers. After successful en bloc surgical isolation of the pulmonary veins and posterior left atrium, postoperative testing showed inducible AF only in the isolated posterior left atrium but not the remaining of the atrium.[78]

Experimental studies of the posterior left atrium have demonstrated how regions of fibrosis could impede wave-front propagation leading to conduction delays and wave-front division into smaller wavelets.[79, 80] Using noncontact mapping techniques, Markides et al. was first to demonstrate a line of functional block in the posterior left atrium between the two sets of pulmonary veins that correlated with a change in sub-endocardial fiber orientation. This

line of block contributed to AF promotion by local re-entry mechanism and causing wave-front to divide into smaller wavelets.[81] This observation was subsequently confirmed by Roberts-Thomson and co-workers using contact mapping in patients undergoing open heart surgery. They described marked anisotropic conduction with slower conduction perpendicular to the line of block which was more pronounced in those with cardiac failure or mitral regurgitation.[82]

1.2.4 Rotors with Fibrillatory Conduction

The first experimental evidence that AF can be maintained by a single re-entrant circuit came from the work of Schuessler et al. in an isolated canine atrium.[83] During acetylcholine induced AF, the number of re-entrant circuits increased in a dose-dependent fashion initially before stabilization to a small single re-entrant circuit, which was not associated with any anatomic obstacles but resulted in fibrillatory conduction to the rest of the atrium. Subsequently, a number of basic researchers have described localized regions of high-frequency activity with spatio-temporal periodicity "driving" AF.[84-87] Similar observations were also made from a number of clinical studies.[75, 88-91]

These rotors tend to anchor to sites with anatomical heterogeneity such as the posterior left atrium or the pulmonary vein ostia of the patients with

paroxysmal AF.[75, 86, 91] However, in the patients with persistent AF, sites of high frequency were more likely to be found in other left atrial locations.[75, 92, 93] A left to right activation gradient has also been shown indicating that the sources or rotors maintaining AF originated from the left atrium.[75, 91, 94, 95] The clinical relevance of these rotors can be seen from the effects following catheter ablation. In a rapid atrial paced canine model, Morillo and co-workers demonstrated that cryoablation at the left atrial sites with shortest AF cycle length resulted in restoration of sinus rhythm.[96] In the clinical setting, retrospective analysis by Sanders et al. showed that ablation at sites of high frequency resulted in prolongation of the AF cycle length and AF termination.[91] More recently, real time high frequency mapping guided AF ablation has been shown to be associated with long term sinus rhythm maintenance.[97]

1.2.5 Summary

The last century has witnessed a significant evolvement of our knowledge regarding the mechanisms initiating and maintaining AF. It appears that in the individual patient with AF, multiple mechanisms can be responsible especially if the underlying atrial substrate is abnormal and dynamic due to the presence of changing co-existing conditions. The limitation of our understanding has thus far limited our ability to cure this condition. Indeed, more research is still required to narrow this knowledge gap regarding AF and its mechanisms.

1.3 Tachycardia related Atrial Remodeling

1.3.1 Atrial Electrical Remodeling

Epidemiological data had suggested that AF is a progressive disease whereby the transition from paroxysmal to chronic AF occurred more frequently in those with longer episodes of this arrhythmia and those with underlying cardiovascular disease.[98] This domestication of AF was also supported by the observations that cardioversion of this arrhythmia became less successful and the chance of arrhythmia recurrence higher with longer duration of AF.[99-101] However, it was the seminal work presented by Wijffels et al. in 1995 on tachycardia related atrial remodeling in chronically instrumented goats that popularized the concept of 'AF begets AF'.[102]

1.3.1.1 Atrial Refractoriness

In this goat model of artificially maintained AF, susceptibility to AF progressively increased from within the first 24 hours of induced AF, together with a progressive increase in the duration of AF to become more sustained after longer periods of the arrhythmia.[102] In this study, the shortening of atrial ERP was accompanied by the loss of normal rate adaptation with less shortening observed at higher pacing rate. Similar changes were also published by Morillo

and colleagues in a canine rapid atrial pacing model at the same time.[96] Further experimental confirmations of these findings were subsequently presented by multiple other investigators.[103-109] Additional emphasis was placed on the increased ERP heterogeneity as an independent determinant of AF inducibility and duration in one study. [106]

Tachycardia related shortening of the ERP has also been documented in multiple human studies.[108, 110-115] However, Attuel and co-workers were first to associate maladaptation of ERP with increased propensity to atrial arrhythmia in humans.[116] Increased dispersion and poor adaptation of cellular ERP was reported by Boutjdir and co-workers in isolated human right atria tissues.[117] Misier et al. also found increased ERP heterogeneity in patients with lone atrial fibrillation undergoing cardiac surgery.[118] One study in chronic persistent AF patients suggested that in addition to ERP heterogeneity, the pattern of ERP dispersion may be important in the predisposition to AF.[119]

1.3.1.2 Fibrillatory Intervals

Due to the presence of an excitable gap during AF, measurement of ERP is feasible although difficult, with investigators also demonstrating entrainment of AF.[120-124] However, fibrillatory intervals have been found to correlate with atrial refractoriness in humans, thereby providing alternate estimates of ERP when direct measurement is difficult.[124, 125] As with the changes seen in atrial

refractoriness, AF results in shorter fibrillatory intervals with increased dispersion.[118, 126-128] In addition, shorter and more disorganized fibrillatory intervals have been observed in the left atrium as compared to the right atrium with chronic AF in a canine study.[72] Capucci et al. also demonstrated the dynamic nature of fibrillatory intervals with prolongation seen prior to termination and shortening seen with AF persistence.[124]

1.3.1.3 Atrial Conduction

While shortening of atrial refractoriness results in shorter wavelengths capable of promoting AF, additional factors may play a role in the development of chronic AF. This was indicated from the work of Wijffels and co-workers when a disparity was observed in the time course of changes in ERP and the development of chronic AF, whereby mean fibrillatory intervals stabilized within days of AF and a few weeks were required for AF to become more sustained.[102] Conduction velocity forms the other factor in the equation since its product with refractoriness determines the wavelength. Even though conduction velocity was unchanged within the first days of AF from initial observations,[102] other investigators found evidence of conduction slowing which contributed to increased AF following stabilization of ERP.[103, 105] Similar time course of changes in ERP and conduction was also observed following the onset of atrial flutter.[109]

Measurement of conduction velocity in clinical studies is often harder without high density mapping. Thus, indices such as P wave duration, linear conduction between two points and presence of electrogram fractionation are used as surrogate measures. Early work by Cosio et al. had demonstrated extra-stimulus induced intra-atrial conduction delays in AF patients.[129] Studies in patients following cardioversion from chronic AF have also shown prolonged P wave duration, longer conduction times and more fragmented atrial activity.[108, 111] Similar changes in conduction properties were also confirmed in more recent evaluation using 3-D electroanatomical mapping systems capable of determining wavefront propagation velocity in human AF.[130]

1.3.1.4 Sinus Node Function

In addition to the changes in ERP, fibrillatory intervals and conduction velocity, sinus node dysfunction has been described in tachycardia related atrial remodeling. In the experimental setting, Elvan et al. demonstrated significant prolongation of corrected sinus node recovery time and sinus cycle length following 2 to 6 weeks of rapid atrial pacing in dogs.[103] Likewise, short duration of atrial pacing in humans has been associated with impaired sinus node function.[131] Further clinical evidence of this abnormality has been reported by various investigators when studying patients following reversion to sinus rhythm from AF or atrial flutter.[111, 114, 132-134] However, the mechanism by which sinus node remodeling due to atrial tachycardia contributes to maintenance of

the arrhythmia remains poorly understood. Perhaps the increased time window and ERP dispersions seen in patients with sinus node dysfunction result in a milieu more vulnerable to develop AF.[135]

1.3.2 Atrial Ionic Remodeling

Electrical changes described in the previous section due to AF may be a reflection of underlying ion channel alterations. Many atrial ion currents have been studied in AF.

1.3.2.1 Calcium

Inactivation or downregulation of L-type calcium current (I_{ca}) is pivotal in ionic remodeling due to AF. I_{ca} contributes to the plateau phase of the action potential. Patch clamp studies of single atrial myocytes from patients with chronic AF showed ~70% reduction in I_{ca} as compared to those in sinus rhythm.[136, 137] Similar reduction in I_{ca} has been demonstrated in the rapid atrial paced canine model, which accounted for the decrease in action potential duration and its maladaptation to rate reflective of the changes in atrial refractoriness due to tachycardia related remodeling.[138] Transcriptional downregulation of the alpha 1c subunit of I_{ca} is thought to be responsible for reduced mRNA concentrations measured in different pre-clinical and clinical studies.[139-142] Additional subunits of I_{ca} – alpha 2/delta 1 and beta 1b, have since

been found to be reduced in human chronic AF also contributing to the reduced I_{ca} amplitude.[143] Of note, the decrease in I_{ca} is not seen in patients with non-persistent AF.[144, 145]

However, the signaling mechanism underlying tachycardia related ionic remodeling remains poorly understood. This reduction in I_{ca} is likely to be an adaptive response to prevent intracellular calcium overload as a result of atrial tachycardia.[146] This theory is based on the findings that inhibition of calcium entry with Verapamil significantly attenuated shortening of ERP and reduced AF inducibility with short term rapid atrial pacing.[147, 148] Because I_{ca} antagonists have been found to be ineffective against remodeling due to longer term tachycardia (>24 hours), other as yet unknown signaling mechanisms are likely to be involved.[149, 150] Moreover, the signaling mechanisms leading to tachycardia related calcium overload causing alterations in ion channel expression remains unknown.

Other calcium related abnormalities include: impaired cellular calcium handling with decreased systolic calcium transients, which have been demonstrated in rapid paced canine atria contributing to impaired cellular contractile function;[151] and increased spontaneous calcium release manifested as calcium sparks and calcium waves in atrial myocytes from patients with AF from the work of Hove-Madsen et al. which is potentially arrhythmogenic.[152] This diastolic calcium leak

from the sarcoplasmic reticulum could be accounted for by protein kinase A hyperphosphorylation of the cardiac ryanodine receptor which may play a role in initiating or maintaining AF.[153]

1.3.2.2 Potassium

The outward potassium currents include: transient outward potassium current (I_{to}); and sustained outward potassium current (I_{Ksus}) consisting of ultra rapid delayed rectifier (I_{Kur}) and the rapid and slow delayed rectifiers (I_{Kr} and I_{Ks}). Together, they are accountable for the repolarization of the action potential. To date, available information suggests a reduction in I_{to} from both pre-clinical and human AF.[136, 138, 154] However, changes in mRNA and protein levels of Kv4.3 (putative gene encoding I_{to}) have been variable across different animal models and subtypes of human AF.[140, 141, 155, 156] Likewise, changes in I_{Ksus} and its components (I_{Kur}, I_{Kr} and I_{Ks}) have been variable without any conclusive findings.[136, 141, 154-157]

The inward rectifying potassium currents I_{K1} and I_{KAch} act to maintain the action potential plateau. Studies have demonstrated unchanged or increased levels of I_{K1}.[136, 138, 154, 158] In contrast, I_{KAch} or its mRNA and protein expressions have been found to be reduced in chronic AF, although one initial study showed the opposite.[136, 155, 156, 158]

1.3.2.3 Sodium and Others

Present understanding regarding sodium current and other exchangers is much more limited as compared to that of calcium and potassium currents. The sodium current (I_{Na}) is the major contributor to the upstroke of the action potential during phase 0 of depolarization. Experimental studies suggest I_{Na} or the mRNA and protein expression levels remain unchanged or decreased with sustained atrial tachycardia, resulting in reduced conduction velocity contributing to the substrate for AF.[140, 141, 159, 160] However, clinical evidence is contradictory to pre-clinical findings with unchanged or increased I_{Na}.[136, 156]

Blockade of the sodium-calcium exchanger (NCX) in canine acute AF model prevented shortening of ERP suggesting a possibly important role of NCX in AF,[161] although protein expression levels of NCX have been variable in different studies.[140, 162] Blockade of the sodium-hydrogen exchanger (NHE) prevented short term but not long term tachycardia related atrial remodeling in canine studies. [163, 164] Although it may appear to have a role in preventing contractile dysfunction, no effect was seen with electrical remodeling in the goat model.[165, 166]

1.3.3 Atrial Structural Remodeling

Initial observations by Wijffels et al. in their goat study showing that AF only became more sustained a few weeks following the completion of electrical remodeling, led to the conclusion that other factors are involved in the development of a substrate for AF.[102] Indeed, study of pacing induced AF periods of 1 month separated by 1 week of sinus rhythm in the goat model demonstrated the cumulative effect of AF on its inducibility and stability although this was not seen in a prior study with 5 days of AF paroxysms separated by 2 days of sinus rhythm.[167, 168] Since complete reversal of electrical remodeling has taken place in between these AF episodes, the presence of a "second factor" independent of electrical changes was therefore suggested. Recently, Stiles et al. reported significant structural abnormalities characterized by loss of myocardial voltage in conjunction with electrical changes of conduction slowing, altered sinus node function and increased atrial refractoriness in paroxysmal lone AF patients as likely contributors to the "second factor" that predisposes to the development and progression of AF.[169] This "second factor" contributing to more sustained AF has been studied extensively by various investigators.

1.3.3.1 Atrial Myocytes – Degeneration, De-differentiation or Apoptosis?

Significant structural changes seen in atrial myocytes from humans or experimental AF models are most likely due to degeneration or de-differentiation.[96, 170-175] These changes differ across species and models, but can include the following:

i. Cellular hypertrophy
ii. Disruption of the sarcoplasmic reticulum
iii. Changes in mitochondrial size and shape
iv. Widening of intercalated discs
v. Central loss of sarcomeres (myolysis)
vi. Peri-nuclear glycogen accumulation
vii. Dispersion of nuclear chromatin
viii. Changes in structural cellular proteins

Signs of irreversible changes leading to cellular apoptosis or abnormalities in the apoptotic markers such as bcl-2, p53, proliferating nuclear antigen and TUNEL reactivity were not found in chronic lone AF patients or experimental AF models.[172, 174, 176] Of note, evidence of programmed cell death has only been documented in one human study of patients with AF and concomitant atrial dilatation due to co-existing valvular or coronary artery disease.[177] In these patients with associated cardiac diseases, structural changes have been noted to be more extensive than patients with lone AF.[177-180]

1.3.3.2 Gap Junctions

Gap junctions consist of various proteins from the connexin family forming direct cytoplasmic continuity between cells to provide cell to cell electrical coupling. Hence, gap junction abnormalities in quantity and distribution can affect conduction velocity and anisotropy leading to increased re-entry.[181, 182] Atrial myocytes contain 3 different isoforms of connexin: connexin 40, connexin 43 and connexin 45, with significant heterogeneity in their distribution across cardiac chambers and animal species.[183, 184] This heterogeneity is a likely contributor to the conflicting results we have seen to date regarding the role of connexin in AF related remodeling. Other contributors can include: shortcomings in the analytical methods of western blotting and immunofluorescence confocal analysis, and the complex heteromeric (channels containing different connexin isoforms) or heterotypic (differential channel interactions) makeup of atrial gap junctions.[185] With this knowledge in mind, the remodeling of different connexin isoforms in AF is hereby explored.

Connexin 40: Evidence from transgenic mice studies suggested a link between connexin 40 deficiency and increased conduction abnormalities with increased AF vulnerability.[186-188] From the goat model of AF, van der Velden and co-workers have shown that chronic AF was associated with increased heterogeneity of connexin 40 distribution which correlated with the time course

of pacing duration and stability of AF, although its quantification had remained unchanged in one study and decreased in another.[189, 190] This increased distributional heterogeneity has also been reported in human studies but the quantity of connexin 40 was either increased or decreased as compared to controls. It is uncertain whether these changes are attributable to AF alone as the patients studied had various underlying cardiac diseases.[191-193] Nevertheless, one clinical study has shown increased concentrations of connexin 40 in the left atria of lone AF patients undergoing surgical radiofrequency ablation of AF.[194] In addition, lateralization of connexin 40 has also been described in human studies but the significance of which remains unknown.[192, 195] Finally, somatic mutations in the connexin 40 gene (GJA5) and connexin 40 polymorphism have recently been identified in patients with lone AF. [196, 197]

Connexin 43: Different animal models of AF have yielded differing quantities of connexin 43 expressions with unchanged levels in the goats and increased levels in the dogs.[189, 190, 198, 199] Similar variability was also observed in human studies of patients with chronic AF and concurrent cardiac diseases.[191-195] Of note, connexin 43 concentration was unchanged in lone AF patients.[194] Likewise, lateralization of connexin 43 has also been reported.[192, 195]

Connexin 45: This connexin isoform is expressed in the atria but at low levels. Limited data is available regarding its role in AF related remodeling. However, its level was unchanged in one study on patients with postoperative AF.[191]

1.3.3.3 Atrial Interstitial Fibrosis

Atrial interstitial fibrosis is a hallmark of structural remodeling commonly seen in patients with AF, often as a result of underlying co-morbidities such as old age, hypertension, congestive heart failure, valvular heart disease and myocardial ischemia. Fibrosis whether reparative or reactive, can impair intercellular coupling. By interfering with wavefront propagation due to local conduction slowing or block, it causes increased conduction heterogeneity resulting in an arrhythmogenic substrate.[192, 200] Increased atrial fibrosis has been documented in rapid pacing induced AF in different animal models.[87, 201] Frustaci et al. in their study in lone AF patients also found increased atrial interstitial fibrosis from endo-myocardial biopsy specimens.[171] Likewise, Boldt et al. demonstrated increased collagen I but not collagen III or fibronectin in lone AF patients as compared to patients in sinus rhythm.[202] However, the precise mechanisms underlying atrial interstitial fibrosis are not fully elucidated.

Currently, the known profibrotic factors include angiotensin II and transforming growth factor-beta 1. In transgenic mice with cardiac restricted angiotensin-converting enzyme, Xiao and co-workers demonstrated that increased

angiotensin II was associated with marked bi-atrial enlargement with focal fibrosis and increased AF.[203] Also, treatment with angiotensin converting enzyme inhibitor or angiotensin receptor blockers have been found to reduce atrial fibrosis, its related conduction abnormalities and AF inducibility in rapid paced canine atria.[204, 205] Similarly, Boldt et al. demonstrated reduced collagen I in lone AF patients treated with angiotensin converting enzyme inhibitor.[206] In addition, Verheule et al. showed significantly increased conduction heterogeneity associated with increased AF vulnerability in fibrotic atria of transgenic mice overexpressing transforming growth factor-beta 1.[207]

1.3.4 Atrial Mechanical Remodeling

The term tachycardia-induced atrial cardiomyopathy was first used to describe the atrial electrophysiological and anatomical remodeling associated with AF.[208] However, much like the well recognized entity of tachycardia related ventricular cardiomyopathy; there is now evidence of impaired atrial mechanical function as a result of atrial tachycardia, which is also known as atrial mechanical remodeling. Importantly, it is this loss of atrial contractile function that predisposes to atrial thrombus formation and increased risk of thromboembolic stroke.

Observations from animal studies have demonstrated significant reduction in left atrial function and left atrial appendage emptying velocity even within hours of rapid atrial pacing.[165, 209-211] Similar findings were also reported in a short term rapid atrial pacing study in humans by Daoud et al.[212] The effect on atrial mechanical remodeling due to longer duration of AF can be derived from post cardioversion studies in chronic AF patients. Early observations made in the 1960s by Logan et al. of the loss of 'a wave' in the atrial pressure curve following cardioversion of AF, associated the loss of contractile function with this arrhythmia.[213] This was later confirmed by other investigators, who by using echocardiography observed that the degree of mechanical dysfunction was greater with longer duration of AF.[214-217]

Current understanding on the mechanisms underlying tachycardia mediated atrial mechanical remodeling remains incomplete. In the post cardioversion studies, atrial mechanical dysfunction was seen independent of the mode of cardioversion; be it spontaneous reversion, pharmacological or electrical, although a varying degree of mechanical dysfunction has been reported.[210, 218-224] Therefore, the mode of cardioversion is not an important contributor to atrial mechanical remodeling due to tachycardia. A number of possible cellular mechanisms have been observed from different studies including: impaired cellular calcium handling [151], intracellular calcium overload [209, 212], alterations in L-type calcium currents or sodium-calcium exchanger [162, 180], hibernation of

atrial myocytes [170], and myolysis.[180] In addition, Sanders et al. have shown that atrial contractile apparatus was intact with short durations of atrial arrhythmias whereby complete reversal of mechanical function was attainable with pacing at higher rates or administration of calcium or isoprenaline.[217, 225] In contrast, the response to increased stimulation rate and isoprenaline was attenuated in those with longer duration of AF. This led to the postulation that additional mechanisms such as underlying structural abnormalities could account for the mechanical remodeling due to more prolonged arrhythmia.[217]

1.3.5 Time Course of Remodeling

Tachycardia related atrial remodeling has been found to occur at a different time domain. Electrical remodeling occurs within the first few days of AF and goes "hand in hand" with contractile remodeling.[102, 211] In contrast, structural remodeling occurs progressively but much later, from weeks to months of AF and may contribute to the development of persistent AF.[174, 226] Importantly, reverse electrical remodeling has been shown to occur within a few days while recovery from mechanical and structural remodeling required a much longer period of time.[102, 173, 211, 214, 219, 227, 228] In addition, multiple studies have demonstrated complete reverse electrical remodeling even after prolonged AF as compared to partial recovery of structural changes.[108, 173, 228, 229] However, the time course of reverse electrical remodeling varied according to the duration of

AF while also exhibiting regional differences.[107, 115, 230, 231] The differential time course of tachycardia related atrial remodeling provides additional understanding on the mechanisms at play during acute and chronic AF. It also highlights the importance of structural changes in contributing to the chronicity of AF and hence of appropriate early intervention to prevent structural remodeling.

1.3.6 Summary

Since the initial studies on tachycardia related atrial electrical remodeling, investigators have explored the associated ionic, structural and mechanical changes to different extent over the last decade. However, it remains clear that our understanding of the underlying mechanisms in atrial remodeling is incomplete. Nevertheless, the extensive characterization of tachycardia related atrial remodeling has provided an important foundation and basis for comparison in subsequent studies on atrial remodeling, due to various clinical substrates for AF which are further explored in Section 1.8.

1.4 Inflammation in Atrial Fibrillation

Recent studies have established a causal link between inflammation and the initiation and maintenance of AF.[232] However, current available literature cannot differentiate whether inflammation is an initiator or a perpetuator of AF,

nor can we be sure whether inflammation is just a response to underlying AF. During the late 1990s, the observation of increased AF post cardiac surgery and its correlation with elevated C-reactive protein level first raised the possibility of an inflammatory link.[233, 234] At about the same time, Frustaci and co-workers' study in lone AF atria demonstrated increased lymphomononuclear infiltrates which were also suggestive of an inflammatory process.[171] Histological evidence of inflammatory infiltrates has also been shown in various animal models of ventricular tachypacing induced heart failure, mitral regurgitation and sterile pericarditis.[235-237]

Moreover, C-reactive protein level was found to be elevated in AF patients where stepwise C-reactive protein elevation was associated with higher AF burden.[238] AF patients with higher levels of C-reactive protein had a greater chance of AF recurrence after cardioversion while C-reactive protein may also assist in predicting patients at increased risk for future development of AF.[239, 240] C-reactive protein level has been found to decline following catheter ablation in patients with AF.[241] Dernellis and colleagues also demonstrated the beneficial effects of methylprednisolone in reducing C-reactive protein levels resulting in reduced recurrence or progression of AF in patients with persistent AF.[242] Likewise, Halonen et al. also showed the beneficial effect of hydrocortisone in a double blind randomized study in reducing the incidence of post-operative AF.[243] Beneficial effects of prednisolone have also been shown in canine

tachycardia-related atrial remodeling.[244] Thus, the reduction in AF by anti-inflammatory measures further substantiated the role of inflammation in AF.

1.5 Autonomic Nervous System and Atrial Fibrillation

The autonomic nervous system has been found to play an important role in both the initiation and maintenance of AF. Anatomically, concentrations of the intrinsic cardiac autonomic nerves are found to converge in the epicardial fat pads, forming the ganglionated plexi located around the great vessels including the pulmonary veins.[245] Various investigators studying the neural basis of AF have examined both vagal and sympathetic effects on atrial electrophysiology.

1.5.1 Vagal Effects on Atrial Electrophysiology

Early observations made by Coumel et al. of sinus slowing preceding the onset of atrial arrhythmia led to their hypothesis that vagal activity might predispose the development of atrial arrhythmia.[246, 247] The times with heightened vagal tone are during rest, night-time or post-prandially. The electrophysiological consequences of increased vagal activity include: heterogeneous ERP shortening, variable atrioventricular conduction, shortening of wavelength and greater AF inducibility/stability.[248-253] This combination of factors suggests a possible re-entry mechanism leading to increased AF.

1.5.2 Sympathetic Effects on Atrial Electrophysiology

Tan and colleagues showed in a canine study that heightened sympathetic tone can lead to increased atrial arrhythmia due to increased focal discharges from sites with abundant sympathetic innervations and conduction tissues such as the thoracic veins.[254] The capability of automaticity and triggered activity in thoracic veins from sympathetic stimulation has also been shown by Wit et al.[255] More recent works have suggested the role of elevated diastolic calcium transients and subsequent early after-depolarizations in causing triggered arrhythmia.[256, 257] However, sympathetic stimulation has been found to be less effective than vagal stimulation in promoting AF even though similar shortening of ERP was seen.[250, 258] In one study, this has been attributed to its lack of effect on ERP heterogeneity.[250]

1.5.3 Neural Modulation in Atrial Fibrillation

Although favorable experimental results with radiofrequency ablation of ganglionated fat pads were obtained by various researchers,[198, 259, 260] Hirose et al. found increased AF inducibility in their canine work.[251] Likewise, clinical studies have yielded conflicting results.[261-264] Of note, there is limited data available regarding the safety and efficacy of ablation strategy targeting the autonomic nervous system. The major issues regarding catheter ablation of the cardiac autonomics include accurate localization of these ganglions, the

technique of effective elimination and the need of a good randomized protocol to assess its true role.[265]

1.5.4 Summary

It was postulated that vagal influences predominate in patients with structurally normal hearts, while sympathetic influences play a greater role in those with structural heart disease.[246] However, it is likely that a complex interplay between both parts of the autonomic system exists in contributing to AF initiation and maintenance. In particular, there is further evidence of initial increased sympathetic stimulation followed by heightened vagal response in different types of AF.[266-268] However, at present, adjunctive neural modulation strategy in catheter ablation of AF cannot be recommended until further results become available.

1.6 Stretch and Atrial Fibrillation

Atrial stretch with resultant atrial dilatation often co-exists in a number of clinical substrates predisposing to the development of AF. Its mutual dependency with AF is well established given it is known as a cause as well as consequence of AF. To date, the underlying arrhythmogenic effects have been explored in both animal and human studies, in the absence and presence of co-

existing conditions. Atrial remodeling in different models of acute and chronic stretch is hereby explored.

1.6.1 Cause and Consequence of AF

Since the 1950s, bi-atrial enlargement has been recognized to correlate with increased AF in patients with valvular heart disease.[269, 270] Various large population studies followed in the 1990s confirming the independent association between left atrial size and the development of AF.[271-273] Specifically, data from the Framingham Study estimated that for every 5mm increment in left atrial dimension, there was a 39% increase in the risk of AF.[271] Taken together, these data suggest a possible role of atrial dilatation in causing AF.

In addition, other studies implicate that AF itself can result in atrial enlargement.[274-277] Probst et al. reported in the early 1970s that left atrial enlargement was secondary to AF rather than causing it.[274] Sanfilippo et al. have shown in a prospective echocardiographic study, evidence of significant bi-atrial enlargement due to AF in patients with no evidence of underlying structural heart disease and initial normal left atrial size.[275] More recently, Dittrich et al. estimated that AF independently accounted for approximately 2.5mm increase in left atrial diameter in patients with non-valvular AF.[276] Other studies in lone

AF patients did not find any significant change in left atrial dimension over a 20 to 30 month follow-up period, which led to the postulation that left atrial enlargement occurs only with longer AF duration.[278, 279] Although it remains unknown whether atrial enlargement could also be due to other factors, the above findings imply that it can be a consequence of AF.

1.6.2 Atrial Remodeling due to Acute Stretch

Different experimental models of acute stretch have been used to study the effect of atrial dilatation on electrophysiological properties. To date, conflicting results have been demonstrated from both animal and human studies.

1.6.2.1 Animal Studies

The effect of acute stretch on atrial refractoriness has been found to be variable. In-vivo experiments in canine models yielded abbreviation of atrial refractoriness in one study,[280] and prolongation with increased dispersion in others.[281-283] Furthermore, atrial ERP was unchanged in the goat model of acute volume load.[284] Atrial conduction was found to be significantly slowed by Sideris et al.[281] while all the aforementioned studies demonstrated increased AF inducibility and longer AF episodes.[280-282, 284]

However, atrial refractoriness was found to be consistently decreased in different isolated heart studies. Ravelli and Allessie reported shortening of left atrial ERP with high intra-atrial pressure in the Langendorff-perfused rabbit hearts.[285] Similar shortening of atrial ERP was documented by other investigators.[286] In addition, Nazir and Lab also reported a significant decrease in monophasic action potential at 50% repolarization with acute stretch produced by intra-atrial balloon inflation in the Langendorff-perfused guinea pig hearts.[287] In addition, Ejisbouts and co-workers demonstrated conduction slowing, increased intra-atrial conduction block and increased spatial conduction heterogeneity in isolated rabbit hearts subjected to acute stretch.[288]

1.6.2.2 Clinical Studies

The effect of acute stretch on atrial refractoriness has also shown divergent results. Perhaps, this could be due to different pacing protocol, pacing cycle length, pacing site, degree of atrial pressure increment and autonomic tone in the various studies.[289] Calkins and co-workers initially reported no change in atrial ERP or AF inducibility with simultaneous atrial and ventricular pacing.[290] However, the same group reported a decrease in ERP in a subsequent study using a modified pacing protocol which derived a more acute rise in atrial pressure.[291] Likewise, Tse and co-workers demonstrated ERP shortening with acute increase in atrial pressure which was enhanced by autonomic blockade and attenuated by calcium channel blockade.[292] In contrast, two other studies

reported an increase in atrial ERP with an increase in atrial pressure during simultaneous atrial and ventricular pacing.[293, 294] In addition, increased ERP heterogeneity was also reported by two of the above studies.[292, 294]

1.6.3 Chronic Stretch

Atrial ERP has been reported to remain unchanged or increased in studies on chronic stretch regardless of any underlying co-morbidities.

1.6.3.1 Animal Studies

Initial insights regarding atrial electrophysiological changes due to chronic atrial stretch were gained from the work of Boyden and colleagues. No change in atrial ERP was reported although an increase in connective tissue content was seen in dogs with valvular heart disease.[295, 296] However, increased atrial ERP has been shown in rapid ventricular pacing induced heart failure animals.[200, 297] In particular, Li and co-workers also demonstrated increased atrial conduction heterogeneity and interstitial fibrosis in their dogs with heart failure.[200] Likewise, similar observations were made by Verheule et al. in a canine mitral regurgitation model where ERP was prolonged together with increased AF inducibility and fibrosis but without any change in conduction properties.[298] In addition, Kistler et al. showed increased atrial fibrosis and conduction abnormalities in an ovine model of chronic hypertension although ERP was

unchanged in that study.[299] In another model of chronic stretch due to complete atrio-ventricular block, Neuberger et al. reported that atrial ERP was also unchanged while conduction delays were seen with longer induced AF episodes but without an increase in interstitial fibrosis.[300]

1.6.3.2 Clinical Studies

Atrial refractoriness in control patients without any history of atrial arrhythmia has been found to be longer in those with an enlarged atrium than those with normal atrial size.[301] Further insights can be gained from patients with conditions known to result in chronic left atrial stretch such as congestive heart failure, mitral stenosis and atrial septal defects. Sanders et al. demonstrated increased atrial ERP, conduction abnormalities and propensity for AF in patients with congestive heart failure.[302] Similar findings were reported by John et al. and Roberts-Thomson et al. in patients with severe mitral stenosis and atrial septal defects respectively.[303, 304] These investigators were also able to demonstrate significant underlying atrial structural abnormalities by evidence of increased areas of low voltage or scarring using electro-anatomical mapping system.[302-304] In addition, the work of Sparks et al. in patients undergoing long term asynchronous ventricular pacing also showed significant bi-atrial dilatation and increased ERP.[305]

1.6.4 Underlying Mechanisms

The mechanisms underlying stretch related atrial remodeling may include the following: First, stretch may result in after-depolarizations which can initiate AF by means of mechanoelectric feedback.[287, 306, 307] Second, stretch activated ion channels have been demonstrated to be present in the atrial myocardium.[308-311] They may play a role in stretch related atrial electrical remodeling since inhibitors of these channels such as gadolinium and taruntula have been shown to reduce vulnerability to AF, even though they had no effect on atrial ERP.[312, 313] Moreover, streptomycin, another inhibitor of stretch- activated ion channels, and gadolinium have been shown to suppress stretch related after-depolarizations while streptomycin also reduced ERP shortening.[307, 314] Third, calcium loading may be a potential cellular mechanism since calcium channel blockers have been shown to reduce stretch related AF.[286, 292] Fourth, the autonomic nervous system has been implicated since autonomic blockade facilitated stretch induced AF maintenance with increased ERP heterogeneity.[292] Last, alteration in atrial substrate such as increased atrial size, abbreviation of ERP, conduction slowing and increased conduction or ERP heterogeneity due to acute stretch, may contribute to increased AF due to reentrant mechanism.[285-288, 292, 294]

1.6.5 Summary

Despite the known association between atrial dilatation and AF, studies on atrial remodeling due to stretch have yielded conflicting results due to various possible reasons outlined above. More importantly, a gap still exists in our understanding of the mechanistic link. In addition, it remains unclear whether atrial dimension would be a useful index to direct additional therapy or provide prognostic information in patients with AF and atrial dilatation.

1.7 Complex Fractionated Atrial Electrograms

Complex fractionated atrial electrograms (CFAE) have been implicated to represent a variety of mechanisms capable of sustaining re-entry thereby contributing to the maintenance of AF. Thus, targeting of CFAEs has been increasingly integrated into various catheter ablation protocols.

1.7.1 Definition of CFAEs

The definition for CFAEs varies in different clinical studies:

i. Continuous electrical activity or electrograms with FF interval <100ms;[127]

ii. Fractionated atrial electrograms with a cycle length ≤120ms or with ≥2 deflections or perturbation of the baseline with continuous deflection of a prolonged activation complex;[315]

iii. Fractionated potentials with ≥3 deflections or continuous activity;[316]

iv. Electrograms with a cycle length ≤120 ms or shorter than in the coronary sinus or displayed continuous electric activity.[317]

1.7.2 Mechanisms of CFAEs

A number of possible mechanisms underlying CFAEs have been identified to date. First, CFAEs could represent areas with slow and anisotropic conduction. Work by Gardner and co-workers in healed infarcted canine hearts elegantly correlated fractionated signals with structural changes of fibrosis resulting in reduced cell to cell connections thereby slowing conduction.[318] Likewise, Spach and Dolber made very similar observations in their human atria work.[319] In studying the posterior left atrium, Roberts-Thomson et al. also concluded that fractionated electrograms occurred almost exclusively along the line of conduction block.[82] Second, CFAEs could represent pivoting points causing turning around of propagating wavefronts. This has been shown in humans undergoing Wolff-Parkinson-White surgery by Konings et al. using unipolar electrograms.[320] In addition, using isolated sheep hearts, Kalifa et al. demonstrated that the most fractionated electrograms were found at the

border zone of high frequency activity where the greatest variability in the propagation were observed.[79] Third, CFAEs could result from increased vagal stimulation. This was demonstrated by Lin and co-workers in a canine experimental setup where increasing acetylcholine resulted in increased electrogram fractionation.[321]

1.7.3 The Odyssey of Mapping and Targeting CFAEs

Catheter ablation of AF targeting the substrate of CFAEs has yielded varying results to date. Nademanee and colleagues have demonstrated the best success rates of 81%, at longer term follow-up of more than 2 years with a mean of 1.68 procedures per patient.[315, 322] The sites commonly associated with CFAEs were the inter-atrial septum, pulmonary veins, anterior left atrium, left atrial roof, base of the atrial appendage, mitral annulus and proximal coronary sinus.[315, 323] Unfortunately, this result was not always reproducible in other laboratories even as a hybrid procedure with isolation of the pulmonary veins.[317, 323, 324] This discrepancy highlighted the complexity of modern catheter ablation procedure, whereby factors such as comprehensive mapping of the left atrium, choice of radiofrequency power for ablation in addition to ablation techniques or strategies might influence eventual outcome.

1.7.4 Summary

The theories behind the possible mechanisms of CFAEs are rather robust even though more work is required to improve our understanding. A consistent approach in defining and characterizing fractionation is required. With improved understanding of the mechanisms of CFAEs, improved reproducible and objective automated algorithms to identify these sites and better left atrial mapping accuracy, CFAEs guided ablation is likely to remain on the map of different ablation strategies aiming at curing AF.[325]

1.8 Atrial Remodeling in Common Clinical Substrates

Long term follow-up data from the Framingham Study has identified various independent risk factors for the development of AF. These include aging, hypertension, heart failure, valvular heart disease, diabetes, male gender and myocardial infarction.[326, 327] Other conditions known to be associated with AF include sick sinus syndrome, atrial septal defect and end stage renal failure.[328-330] More recently identified associations include obesity and obstructive sleep apnea.[331-334] Characterization of atrial remodeling in these substrates for AF has provided further insights into the underlying arrhythmic mechanisms. However, not all of the above-mentioned have been examined in detail due to the non-availability of suitable animal models and the presence of multiple co-morbidities in patients that often confound the findings. In addition, it is often

difficult to examine the substrate prior to the development of AF in the clinical setting.

1.8.1 Aging

The prevalence of AF is known to increase with age from an estimated 2.3% in those older than 40 years to 5.9% in those older than 65 years.[335] In addition, the odds ratio for each decade of advancing age was between 2.1 to 2.2.[326] The atrial electrophysiological effects of aging include: Increased ERP, sinus node dysfunction, conduction slowing and anisotropy with increased electrogram fractionation. [319, 336-340] The underlying structural effects of aging include increased interstitial fibrosis with increased regions of low atrial voltage.[341, 342] In addition, Roberts-Thomson and co-workers have demonstrated increased atrial electrogram fractionation which correlated with increased age in regions with low voltage and conduction slowing.[343]

1.8.2 Hypertension

Hypertension is not only predictive of AF but given its high prevalence in the population, it accounts for more AF than any other risk factors.[327] After adjusting for other associated conditions, hypertension was found to increase AF risk by 1.5- and 1.4-fold in both men and women.[327] A number of clinical features have been established as predictors of AF development in hypertensive

subjects: increasing age, left atrial enlargement, left ventricular hypertrophy, higher blood pressure level, increased P-wave duration/dispersion and reduced diastolic mitral flow.[344-346] Specifically, for every 5mm increase in left atrial dimension, the risk of AF increases by 39%.[327, 347] While there has been a lack of direct electrophysiological evaluation to understand the mechanisms for AF development in hypertension, some factors can be deduced from clinical studies. Firstly, slowing of atrial conduction can be postulated from the predictors of AF in increased P-wave duration and dispersion in patients with essential hypertension.[345, 348] Secondly, increased atrial ectopies seen with 24 hour ambulatory monitoring in essential hypertensive patients may be an indication of greater triggers of AF.[349]

The presence of hypertension in AF patients has been shown to further increase risk of stroke, cardiovascular mortality and hospitalizations for heart failure.[350] Despite the clinical links between hypertension and the development of AF, there still remains a paucity of studies on the underlying patho-physiological mechanisms of AF in hypertension. To date, there is only one study by Kistler and colleagues, which described the atrial electrical and structural effects of chronic hypertension exposure of 4 to 5 years duration in a novel ovine model after prenatal corticosteroid exposure.[299] They uncovered widespread conduction slowing with increased conduction heterogeneity, no change in atrial refractoriness, increased atrial fibrosis with apoptosis and evidence of

cellular myolysis which led to increased induced AF durations. However, there has been concern that this unique model may not be representative of human disease.[351]

1.8.3 Congestive Heart Failure

Both congestive heart failure and AF have emerged as epidemics in this new millennium.[3, 352] Heart failure was the strongest predictor for the development of AF in the Framingham Study with the highest relative risk of up to 5.9.[326] The complex causal association and compounding interaction between AF and heart failure often lead to more detrimental consequences as compared to each condition in isolation.[353-355] In addition, in the *Candesartan in Heart failure – Assessment of Reduction in Mortality and Morbidity* (CHARM) program, which enrolled patients with chronic heart failure, AF was associated with increased risk of cardiovascular outcomes in heart failure patients including those with preserved left ventricular ejection fraction.[356] Moreover, the prevalence of AF was found to increase with the severity of systolic heart failure with <10% in those with New York Heart Association (NYHA) functional class I to approximately 50% in those with functional class IV heart failure.[357]

Since the initial description of experimental heart failure by chronic rapid pacing in 1962, the model has been utilized widely in heart failure research.[358] To date,

all animal studies on heart failure related atrial remodeling have been based on this single model of rapid ventricular pacing. Power et al. showed significant increase in AF susceptibility which was related to the rate of increase in left atrial area despite increased refractoriness in the ovine left atria.[297] Li and co-workers demonstrated the promotion of sustained AF by increased atrial interstitial fibrosis and conduction heterogeneity with no changes in atrial refractoriness and conduction velocity in the canine heart failure model. They described "remodeling of a different sort" given the structural changes with the resultant conduction abnormalities were more important in contributing to AF than atrial refractoriness.[200] This importance was highlighted by the attenuation of atrial fibrosis, conduction heterogeneity and AF vulnerability in heart failure dogs treated with Pirfenidone, an anti-fibrotic agent.[359]

Sanders and co-workers were first to characterize in detail the atrial changes in humans with both ischemic and non-ischemic cardiomyopathy. They demonstrated increased atrial ERP, conduction slowing, sinus node dysfunction as well as structural abnormalities by evidence of areas of low voltage and scar using an electroanatomic mapping system.[302] The clinical entity of congestive heart failure constitutes a heterogeneous group where the different underlying causes have been shown to portend different prognostic value.[360] Therefore, despite these findings, our understanding of the patho-physiological mechanisms of AF in heart failure remains limited.

1.8.4 Mitral Valve Disease

Both incompetence and stenosis of the mitral valve are recognized risk factors for AF.[326, 361] In patients with mitral regurgitation, there is a 5% per year risk of developing AF leading to increased morbidity and mortality.[362] Verheule and colleagues demonstrated atrial enlargement with increased ERP, increased conduction heterogeneity and increased fibrosis/inflammation, which contributed to increased AF vulnerability in a canine model of mitral regurgitation.[363] In patients with severe mitral regurgitation due to valve prolapse, Morton et al. also showed unchanged/increased atrial ERP, prolonged P wave duration and conduction delay at the crista terminalis.[364]

Animal studies did not show any difference in action potential duration in dogs with mitral valve fibrosis. However, significant reduction in muscle cell layers and increased connective tissue between hypertrophied cells were noted.[296] Fan et al. showed significantly shorter ERP and sinus node remodeling without changes in conduction velocity in mitral stenosis patients at the time of percutaneous commissurotomy following cardioversion from chronic AF. Atrial refractoriness were found to increase significantly at 3 month follow-up study following commissurotomy.[365] In addition, Soylu et al. demonstrated an acute increase in ERP with decreased dispersion immediately after relief of mitral

stenosis.[366] More recently, John et al. characterized in more detail bi-atrial changes in patients with severe mitral stenosis without a history of AF.[303] They found significant atrial remodeling in left atrial enlargement and lower atrial voltage with scarring associated with site-specific conduction abnormalities. Despite unchanged or increased ERP as compared to controls, atrial remodeling due to mitral stenosis was associated with increased AF.

1.8.5 Atrial Septal Defect

Patients with atrial septal defect are known to have increased risk of atrial arrhythmias. Gatzoulis et al. reported that 19% of patients with haemodynamically significant atrial septal defect had atrial arrhythmia at the time of surgical closure which persisted in 60% of them at up to 3.8 years following surgical intervention.[367] Right atrial electrophysiological evaluations in patients with atrial septal defect without prior history of AF showed modest increases in ERP, conduction delay at the crista terminalis and sinus node dysfunction.[368] Importantly, these conduction abnormalities were found to persist even after closure of the atrial septal defect. Roberts-Thomson et al. studied the left atrial changes in patients with haemodynamically significant atrial septal defect.[304] They reported increased propensity for sustained AF in these patients in the setting of left atrial dilatation and reduced voltage with

increased left atrial scarring, which were associated with widespread conduction abnormalities despite no change in ERP.

1.8.6 Sinus Node Disease

The association between sinus node disease and AF is well established with the clinical spectrum of sick sinus syndrome characterized by persistent sinus bradycardia, sinus arrest or bradycardia with episodic supraventricular tachyarrhythmias.[328, 369] Long term follow-up of paced patients with sick sinus syndrome showed a progressive increase in the incidence of AF from 7% at 1 year to 28% at 10 years.[370] Initial evaluation of patients with sick sinus syndrome had focused primarily on sinus node function including sinus node automaticity, sino-atrial conduction time and sinus node recovery time under premature atrial stimulations and various pharmacological stress.[371-375] Subsequent evaluations of atrial refractoriness in patients with sick sinus syndrome revealed unchanged or increased ERP with unchanged or greater ERP dispersion.[135, 376-378]

A variety of conduction abnormalities have been reported apart from the changes in ERP. These include slowed atrial conduction as evidenced by P wave prolongation, increased conduction time and presence of greater number/duration of double potentials.[377-379] Sanders et al. also performed right atrial high density electroanatomic mapping in patients with sinus node disease,

which uncovered extensive structural abnormalities consisting of regions with low voltage and spontaneous scarring.[377] The high prevalence of fractionated electrograms in this patient group with particular localization to the high right atrium, suggested more diffuse right atrial structural abnormalities.[377, 380] In addition, these fractionated electrograms were found to be predictive of AF in paced patients with sick sinus syndrome.[381]

1.8.7 Myocardial Ischemia

Both myocardial infarction and angina pectoris are associated with increased risk of AF with relative risk of 3.6 and 2.8 respectively.[7] The onset of AF in acute myocardial ischemia has been known to confer significant negative prognostic impact since the 1970s.[382-384] The pathogenesis of AF in myocardial ischemia is likely to be multi-factorial. Direct evidence from experimental atrial ischemia induced by occlusion of the atrial artery, demonstrated local conduction slowing with re-entry contributing to increased AF. Atrial ERP was unchanged initially but was prolonged following longer duration of ischemia at 5 hours.[385]

Indirectly, cardiovascular effects of myocardial ischemia in hypoxia, ventricular infarction/dysfunction, increased atrial stretch, pericarditis, neuro-hormonal and autonomic nervous system changes are potentially important in contributing to a heterogeneous electrical and structural milieu thereby

resulting in AF.[285, 302, 386-392] Atrial ERP was found to be shortened with loss of rate adaptation following right coronary artery occlusion in a canine study. Although these changes were attenuated by a selective inhibitor of sodium-hydrogen exchanger, atrial arrhythmia was not evaluated in this study.[163] Miyauchi et al. provided further evidence regarding the substrate for AF in chronic myocardial ischemia. They reported higher monophasic action potential duration and amplitude with increased heterogeneity as well as more heterogeneous atrial sympathetic hyperinnervation in association with increased induced AF in dogs 8 weeks following permanent occlusion of the left anterior descending coronary artery.[393]

1.8.8 Obstructive Sleep Apnea

The link between obstructive sleep apnea and AF has emerged more recently with one study estimating this association with an odds ratio of 2.19.[333] This observation was further confirmed by Stevenson et al. in a prospective case series, where a high prevalence of sleep disordered breathing was diagnosed using polysomnography in relatively young patients with AF and normal left ventricular function deriving an odds ratio of 3.04 for this association.[394] The physiologic changes during or as a result of an apneic episode are likely contributors to this association: significant hemodynamic and autonomic

fluctuations, systemic and pulmonary hypertension, sympathetic activation, atrial stretch and inflammation.

To date, only one study has attempted to examine the mechanisms underlying this association.[395] Using a novel animal model of sleep apnea, Ghias et al. demonstrated increased ganglionated plexi neural activity prior to the occurrence of induced AF during apnea, which was prevented by autonomic blockade and neural ablation of the ganglionated plexi. They also reported abbreviation of atrial ERP and increased systolic blood pressure with mechanical alternans during programmed stimulation indicative of both sympathetic and parasympathetic involvement thereby suggesting an autonomic link between sleep apnea and AF

1.8.9 Diabetes Mellitus

Diabetes mellitus has been established as an independent risk factor for the occurrence of AF with an odds ratio of up to 2.1. [326, 396, 397] Recently, a few studies have emerged examining the association these two conditions in small animal models. Kato et al. performed electrophysiological analysis in the Langendorff-perfused hearts from a genetic non-overweight diabetic rat model. They demonstrated increased atrial arrhythmogenicity with intra-atrial conduction disturbance together with diffuse interstitial fibrosis in the diabetic

atria without changes in atrial ERP as compared to controls.[398] In another Langendorff study using streptozotocin-induced diabetic rats, Otake and co-workers concluded that neural remodeling may play a role for increased AF vulnerability in diabetic mellitus. This was based on the findings of decreased ERP with increased heterogeneity and increased AF following sympathetic nerve stimulation in diabetic hearts.[399] However, further work is needed to further elucidate the underlying patho-physiological mechanisms.

1.8.10 Summary

Understanding of atrial remodeling in various clinical conditions not only provides insights into the mechanisms of AF but could potentially identify appropriate novel targets for anti-AF therapies. Significant gap in our knowledge is evident from the above review of atrial remodeling in different clinical substrates. In particular, the effects of obesity and renal failure on atrial remodeling have not been examined to date.

Chapter Two

Characterization of Cardiac Remodeling in a Large Animal 'One-Kidney, One-Clip' Hypertensive Model

2.1 Introduction

Hypertension remains a major cause of cardiovascular diseases contributing to increased morbidity and mortality with increasing prevalence worldwide.[400] It poses an important public health challenge in both developing and developed countries with significant lack of awareness and inadequate treatment.[401-403]

Since the original work of Goldblatt et al in the 1930s with experimental renovascular hypertension, a plethora of other animal models have facilitated advances in high blood pressure research.[404] While no single model can ever be an exact representation of human essential hypertension due to its complex multi-factorial pathogenesis which remains incompletely understood, the various models served to mimic different aspects of hypertension relevant to specific areas of research. Of note, these hypertensive models which include genetic, endocrine, environmental and renal, are mostly available in small animals.[405, 406] The "one kidney-one clip" (1K1C) model is highly attractive as it produces reliable hypertension in a timely fashion which can also be reversed with clip removal.[407]

To date, the 1K1C hypertension model that involved large animals such as dogs and sheep, has only focussed on extra-cardiac characteristics including renal hemodynamics and humoral changes.[408-410] As such, data on the development of hypertension and the associated cardiac anatomical and functional changes in large animals in the absence of concurrent disease remain limited. Therefore, we seek to characterize these cardiac changes in an ovine 1K1C hypertensive model using a combination of cardiac magnetic resonance imaging (CMR) and pathological examination.

2.2 Methods

All procedures were conducted in accordance with the guidelines outlined in the "Position of the American Heart Association on Research Animal Use" adopted on November 11, 1984 by the American Heart Association. Approval for the performance of the study was provided by the University of Adelaide Animal Ethics Committee.

2.2.1 Model Preparation and Study

All animals were housed within individual pens in climate controlled rooms at 22°C. Animals were fasted for 24 hours prior to general anaesthesia which was required for the renal surgeries (both nephrectomy and renal artery clamping),

carotid artery exteriorization and the CMR scans. Intravenous sodium thiopentone (15-20 mg/kg) was used for induction before endo-tracheal intubation while isoflurane (2-4%) in 100% oxygen was utilized for maintenance. Invasive blood pressure, heart rate, pulse oximetry, end-tidal CO_2 and temperature were continuously monitored. Post-operative care after the renal and carotid surgeries included intra-muscular administration of xylazine hydrochloride (0.05 mg/kg) and penicillin for three days.

2.2.2 One Kidney-One Clip Hypertension

Clamping of the renal arteries to induce hypertension has been used since 1934.[404] Amongst different combinations of renal clamping, the 1K1C model has been shown to provide the most reliable development of hypertension in a timely fashion. In our ovine model, unilateral nephrectomy was followed by clamping of the remaining renal artery using custom made Goldblatt-type clamp three weeks later. The degree of induced renal artery stenosis was closely regulated to approximately 60% with a combination of peri-vascular flow (Transonic Flowmeter TS-401 with precision flow probe, Transonic Systems Inc., NY, USA) and Doppler velocity (Acuson XP-128, 7 MHz probe, Siemens Medical Systems, PA, USA) estimations. Blood pressure was measured in the conscious state via cannulation of exteriorized carotid artery, as previously described

(Datascope Passport 2, Datascope Corp., NJ, USA with Transpac II transducer, Abbott Medical, IL, USA).[411]

2.2.3 Renal Surgeries

For both nephrectomy and renal clamping surgeries, lateral positioning of the animals was adopted with vertical incision made about 2 finger breadths anterior to the lateral extent of the paraspinal muscles. The subcutaneous and muscle layers were divided to afford access to the retroperitoneal space. For nephrectomy, the perinephric fat and Gerota's fascia were carefully separated from the surface of the kidney while the renal vessels and ureter were individually ligatured and divided before removal of the kidney.

Renal artery clamping was performed after the animal was allowed 3 weeks to recover. For renal clamping, the renal artery was located by palpation before exposure was undertaken by clearing the surrounding fat. The Goldblatt clamp was then applied under full vision with further adjustment as dictated by the flow and doppler estimation techniques described above. For both surgeries, the wound was closed in layers after ensuring haemostasis at the surgical site. Subcutaneous heparin (5,000 I.U. daily) was administered for three post-operative days to prevent thrombosis in the clamped renal artery. Serum was

collected for measurements of creatinine levels at baseline and just prior to euthanasia.

2.2.4 Cardiac Functional Assessment

CMR (Siemens Sonata 1.5 Tesla MR imaging system, Siemens Medical Solutions, Erlangen, Germany) was utilized to assess both left atrial (LA) and left ventricular (LV) volumes (EDV: end-diastolic volume and ESV: end-systolic volume) and function (EF: ejection fraction) with slice thickness of 6 mm through the atria and 10 mm through the ventricles without any inter-slice gap. The animals were placed and secured in dorsal recumbence position for the CMR scans. Mechanical ventilation was maintained to facilitate ECG-gated image acquisition with adequate breath-holding. All analyses were performed offline using Argus software (Leonardo workstation, Siemens Medical Solutions, Erlangen, Germany).

2.2.5 Pathological Assessment

All animals were euthanized using intravenous overdose of sodium pentobarbital (120 mg/kg) and the hearts promptly removed for structural analysis. Epicardial fat was dissected free from the heart while the gross weight and dimension of relevant chambers were measured. Both atria were immersed fixed with 10% formalin and paraffin embedded for subsequent light

microscopic evaluation. Sections were stained with haematoxylin & eosin and picrosirius red to demonstrate the extracellular matrix.

2.2.6 Statistical Analysis

All continuous variables are reported as mean ± standard deviation and assessed for normality utilizing the Shapiro-Wilk test. All data sets were normally distributed and all comparisons were performed using the Student's t-test. Statistical significance was established at $p<0.05$.

2.3 RESULTS

Twenty three Merino Cross wethers (54±10 kg) were subjected to experimental 1K1C hypertension. All animals survived the renal surgical procedures. Four out of these twenty three (17%) animals were not included for analysis due to premature complications: one developed malignant hypertension on day 11 after renal clamping while three developed heart failure at a mean of 36±6 days. The remaining nineteen animals were followed for a mean of 73±28 (range 40 to 110) days of hypertension. Cardiac anatomical changes of these nineteen animals were evaluated in comparison to twelve size and sex-matched (48±4 kg) controls.

2.3.1 Blood Pressure Profile

Both mean systolic and diastolic BP remained stable from baseline through post unilateral nephrectomy (Systolic BP: 107±12 versus 110±9 mmHg, p=0.2; Diastolic BP: 71±10 versus 68±12 mmHg; p=0.4). However, they both rose during the first week after renal clamping to reach a plateau by four weeks (Systolic BP: 169±27, Diastolic BP: 118±29 mmHg, both p<0.0001; Figure 1). Mean systolic and diastolic BP stayed within a 20 mmHg range between four and fourteen weeks (Systolic BP: 168 to 187 mmHg and Diastolic BP: 118 to 132 mmHg).

2.3.2 Cardiac Anatomical Changes

CMR was undertaken one day prior to euthanasia in a subset of ten out of nineteen hypertensive animals for comparison with the twelve controls. Table 1 presents the changes in cardiac size as determined by CMR. Table 2 presents the gross pathology findings of cardiac chamber weight and dimension. The hypertensive animals demonstrated significant cardiac hypertrophy with increased inter-ventricular septal and posterior ventricular wall thickness as well as increased bi-atrial and left ventricular mass when compared to the controls (Figure 2). Volumetric analysis by CMR revealed significantly larger left atria but preserved LV volume (Table 1).

2.3.3 Cardiac Functional Changes

Within the time frame of the study, left ventricular function was relatively well preserved in the hypertensive group as compared to the controls (LVEF: 42.3±4.7 versus 46.4±4.1%, p=0.1). However, there was significant reduction in left atrial function in the hypertensive animals compared to controls (LAEF: 24.1±3.6 versus 31.6±3.0%, p=0.001) as shown in Table 1.

2.3.4 Serum Creatinine Level

In this ovine 1K1C model, serum creatinine level was preserved from baseline to study endpoint (94±17 versus 96±21 µmol/L, p=0.8).

2.3.5 Histopathological Changes

Light microscopic examination of atria and its appendage showed multifocal degeneration and necrosis of cardiac myocytes associated with increased lymphocytic infiltration and interstitial fibrosis (Figure 3). Picosirius stains showed significant collagen deposition in the extracellular matrix consistent with interstitial fibrosis (Panel B). Myocyte damage was frequently focal and well circumscribed (Panel D), but occasionally extensive (Panel E). Affected myocytes were markedly vacuolated with coagulative necrosis characterized by

swollen, granular or homogeneous, weakly eosinophilic cytoplasm, loss of striations, and nuclear hyperchromasia (Panel E). There was also evidence of substantial lymphocytic infiltration (Panel F).

2.4 DISCUSSION

This is the first study characterizing both blood pressure profile and cardiac changes using state of the art CMR and histo-pathology in a large animal model of hypertension. In this 1K1C model, we found the following:

1) A blood pressure profile where both systolic and diastolic pressures increased after renal clamping to reach a plateau by four weeks;
2) Significant atrial and ventricular hypertrophy;
3) Preserved left ventricular function but significantly reduced left atrial function.
4) Degeneration and necrosis of atrial myocytes with lymphocytic infiltration and interstitial fibrosis.

Importantly, the significant cardiac changes occurred within a relatively short duration of hypertension with preserved serum creatinine levels.

2.4.1 Functional Assessment Using CMR Imaging

The clinical utility of CMR as a non-invasive cardiovascular investigation is growing rapidly. CMR is known to provide high quality information on cardiac

function with a high level of reproducibility such that it is now considered the gold-standard for the in-vivo evaluation of cardiac structure and function. Recently, the pre-clinical utility of CMR in an ovine model of non-ischemic cardiomyopathy has also been described.[412] The convexity of the thorax in the sheep limits the ability of conventional echocardiography to perform complete evaluation of the cardiac chambers. Apart from the superior image resolution, CMR provided additional functional data in the form of the left atrial volumes and ejection fraction. This allowed a more complete characterization of the cardiac functional status in our ovine 1K1C hypertensive model.

2.4.2 Time Course of Cardiac Remodeling in Hypertension

Both the blood pressure profile and degree of left ventricular hypertrophy seen in our model are similar to that reported by other researchers in small animal 1K1C models whereby a 50% increase in blood pressure and 30% increase in LV mass were seen at four to five weeks following renal clamping.[413, 414] However, the finding of early left atrial dysfunction in hypertension is novel and of potential significance as clinical studies have only associated left atrial enlargement as an early sign of hypertensive heart disease.[415] This finding deserves further clinical evaluation and if confirmed, would provide impetus for early treatment of hypertension to prevent “atrial failure” and its consequences such as atrial fibrillation and stroke. In particular, there is evidence of

normalization of left atrial function with anti-hypertensive therapy in hypertensive patients.[416, 417]

2.4.3 Large versus Small Animal Models of Hypertension

It has long been recognised that there is no ideal animal model for hypertension given its complex multi-factorial pathogenesis which remains incompletely understood. In addition, difficulties encountered in experimental models include the inability to produce hypertension at a rate equivalent to human hypertension in the right age group since most models involved younger animals. Nevertheless, animal models have been widely used in the advancement of high blood pressure research into its pathogenesis as well as pharmacological interventions. Amongst the plethora of animal models available, the majority have involved small animals with rats being the most popular species in hypertension research with increasing proportion.[405, 406] While no particular species can mimic all aspects of human hypertrension, large animals have more human-like cardiovascular anatomy, physiology and function which translate into disease manifestations more comparable to those in humans.

2.4.4 Advantages of One Kidney, One Clip Hypertension

The seminal work of Goldblatt et al in the 1930s demonstrated the sustainment of high blood pressure by unilateral constriction of the renal artery while leaving the other kidney intact.[404] Subsequent evolvement of renal clamping experimentations from this original 2K1C design to the 1K1C model has demonstrated a more reliable development of hypertension in a more timely fashion.[407] In addition, given that renovascular hypertension is the most frequent secondary cause of hypertension in humans, the 1K1C model is more physiological than other endocrine or dietary models that involved mineralocorticoids, salt or glucocorticoids and other nephron reduction models. In the absence of genetic large animal models akin to the spontaneously hypertensive rats, 1K1C hypertension presents a highly attractive option.

Furthermore, 1K1C hypertension has been well studied in terms of cardiac and renal hemodynamics together with humoral changes whereby the primary mechanism for hypertension is due to volume expansion from sodium and water retention with no involvement of the autonomic or renin-angiotensin system apart from brief initial renin activation.[410, 414, 418, 419] Importantly, during the chronic phase of hypertension, the 1K1C is unlike the 2K1C model which is angiotensin II dependent and characterized by elevated plasma renin activity.[414,

[420, 421] This difference is of great significance given the important interactions of the renin-angiotensin system in hypertension and cardiovascular homeostasis.

2.4.5 Potential Use of Model

Characterization of the cardiac anatomical and functional changes in this ovine model complements the currently available information on 1K1C hypertension which includes the renin-angiotensin system, systemic and renal hemodynamics as well as peripheral vascular changes. The cardiac remodeling seen in this ovine model is representative of human hypertensive heart disease. The degree of hypertension was well tolerated by the animals except for one with malignant hypertension where systolic blood pressure exceeded 230 mmHg. This model provides a reliable blood pressure profile which is reproducible with an acceptable overall complication rate of less than 20%. It will be useful in various aspects of cardiovascular research relating to high blood pressure.

2.4.6 Study Limitations

The rate and magnitude of blood pressure elevation in this 1K1C model was somewhat accelerated while a more gradual rise might be more physiological when compared to most cases of human hypertension. On the other hand, this might have been a reasonable compromise to ensure greater experimental usability of this model especially for therapeutic studies. CMR scans were

performed in anesthetized and dorsal recumbence state which might have affected cardiac function. Finally, we did not undertake invasive left ventricular hemodynamic characterization of cardiac pressures and function.

2.5 Conclusions

This study characterizes in detail the cardiac anatomical and functional changes in a large animal model of hypertension. This “one kidney-one clip” model reliably produces hypertension with evidence of end-organ manifestations in the heart and can be utilized in wide areas of cardiovascular research. In particular, it can be used to improve our understanding of hypertensive heart disease.

Table 1: Cardiac Magnetic Resonance Characteristics

	Hypertension (n = 10)	Control (n = 12)	P
LA EDV (ml)	42.9 ± 6.8	28.7 ± 6.3	< 0.0001
LA ESV (ml)	32.5 ± 4.9	18.1 ± 4.1	< 0.0001
LA EF (%)	24.1 ± 3.6	31.6 ± 3.0	0.001
LV EDV (ml)	96.6 ± 19.0	85.4 ± 12.8	0.2
LV ESV (ml)	56.0 ± 13.7	45.8 ± 7.8	0.08
LV EF (%)	42.3 ± 4.7	46.4 ± 4.1	0.1

LA, left atrial; LV, left ventricular; EDV, end-diastolic volume;

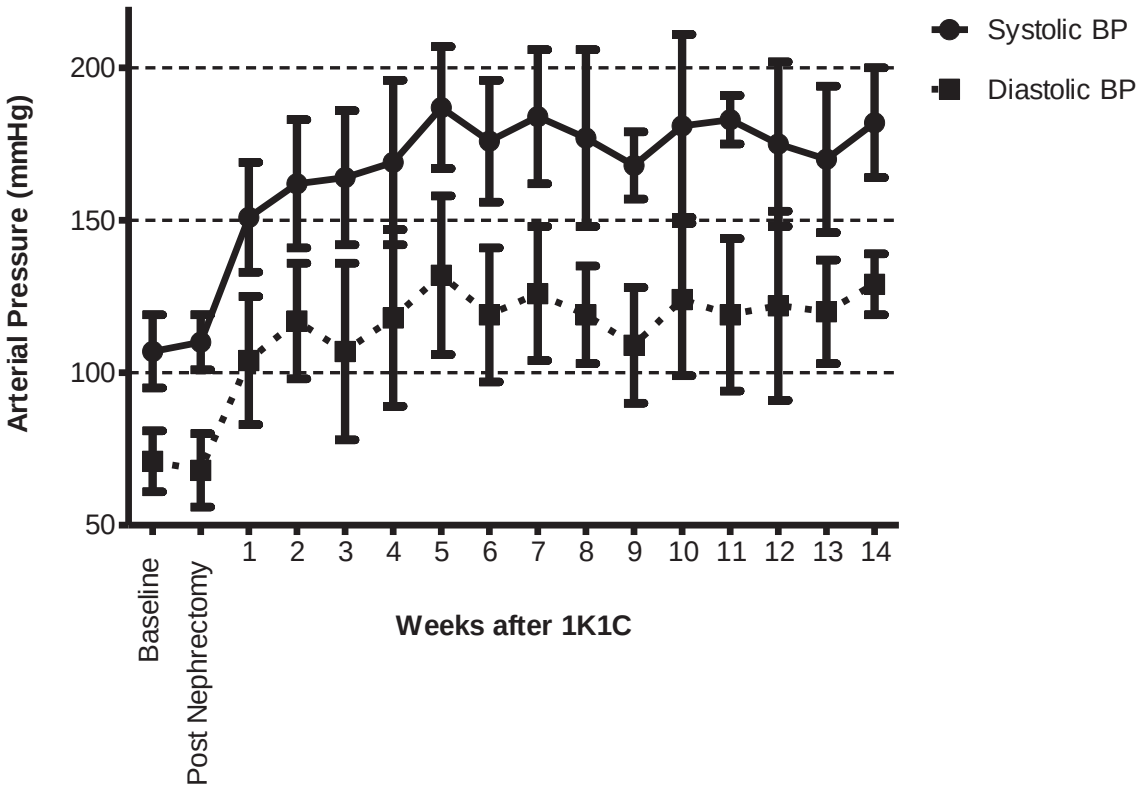
ESV, end-systolic volume; EF, ejection fraction.

Table 2: Cardiac Anatomical Characteristics

	Hypertension (n = 19)	Control (n = 12)	P
LA wt (g)	35.5 ± 6.7	20.9 ± 4.1	0.0003
RA wt (g)	22.9 ± 4.9	15.7 ± 2.8	0.003
IVS wt (g)	58.8 ± 11.4	44.8 ± 5.4	0.007
LV wt (g)	146.3 ± 29.5	98.6 ± 7.3	0.0009
RV wt (g)	63.6 ± 14.1	48.1 ± 4.9	0.01
LV/Body wt (g/kg)	2.7 ± 0.5	2.1 ± 0.2	0.01
IVS (mm)	17.1 ± 1.9	12.2 ± 1.5	<0.0001
PW (mm)	13.6 ± 2.1	10.2 ± 1.2	0.002

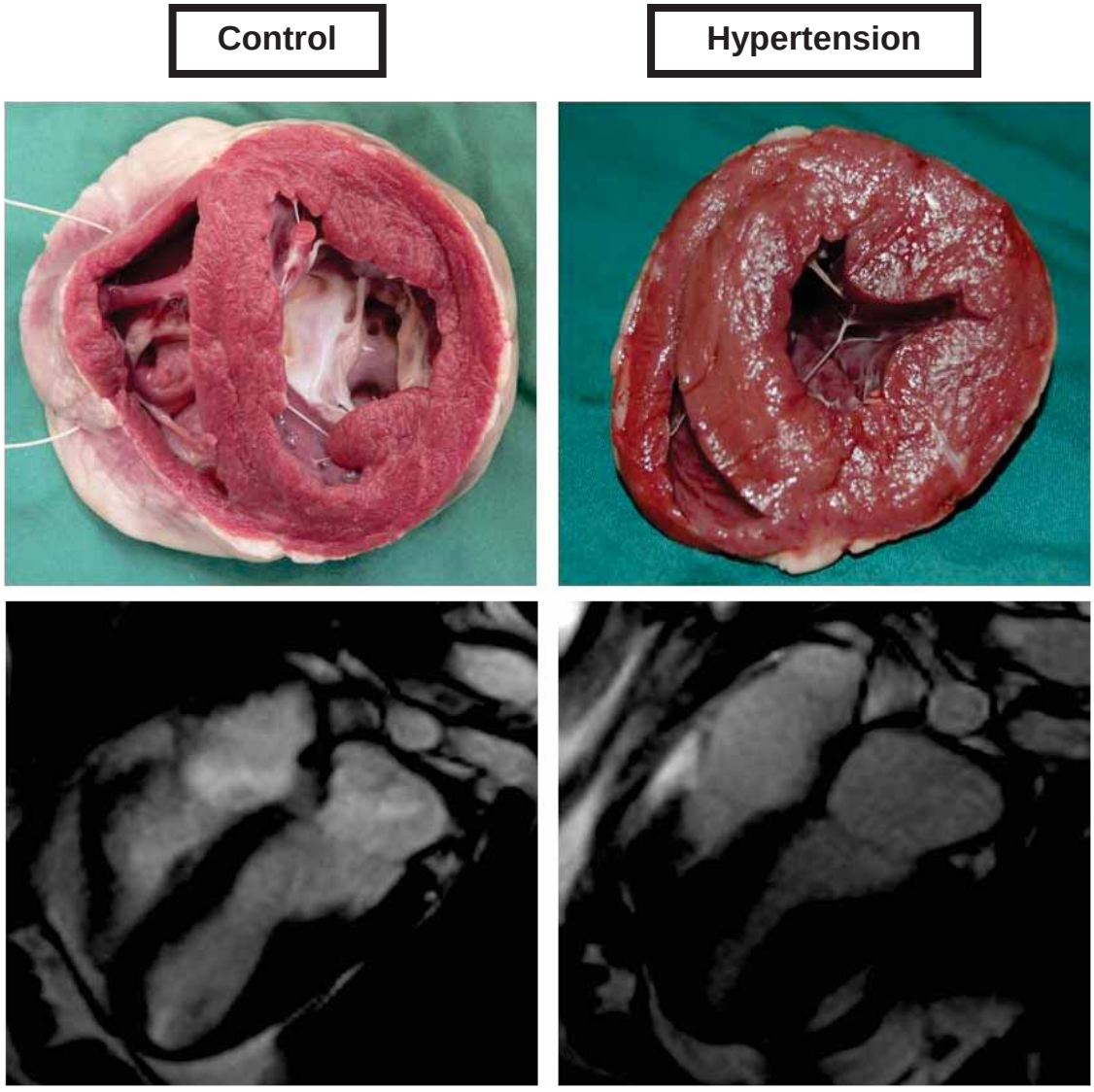
LA, left atrial; RA, right atrial; IVS, inter-ventricular septum; LV, left ventricular; RV, right ventricular; PW, posterior LV wall; wt, weight.

Figure 1: 1K1C Blood Pressure Profile



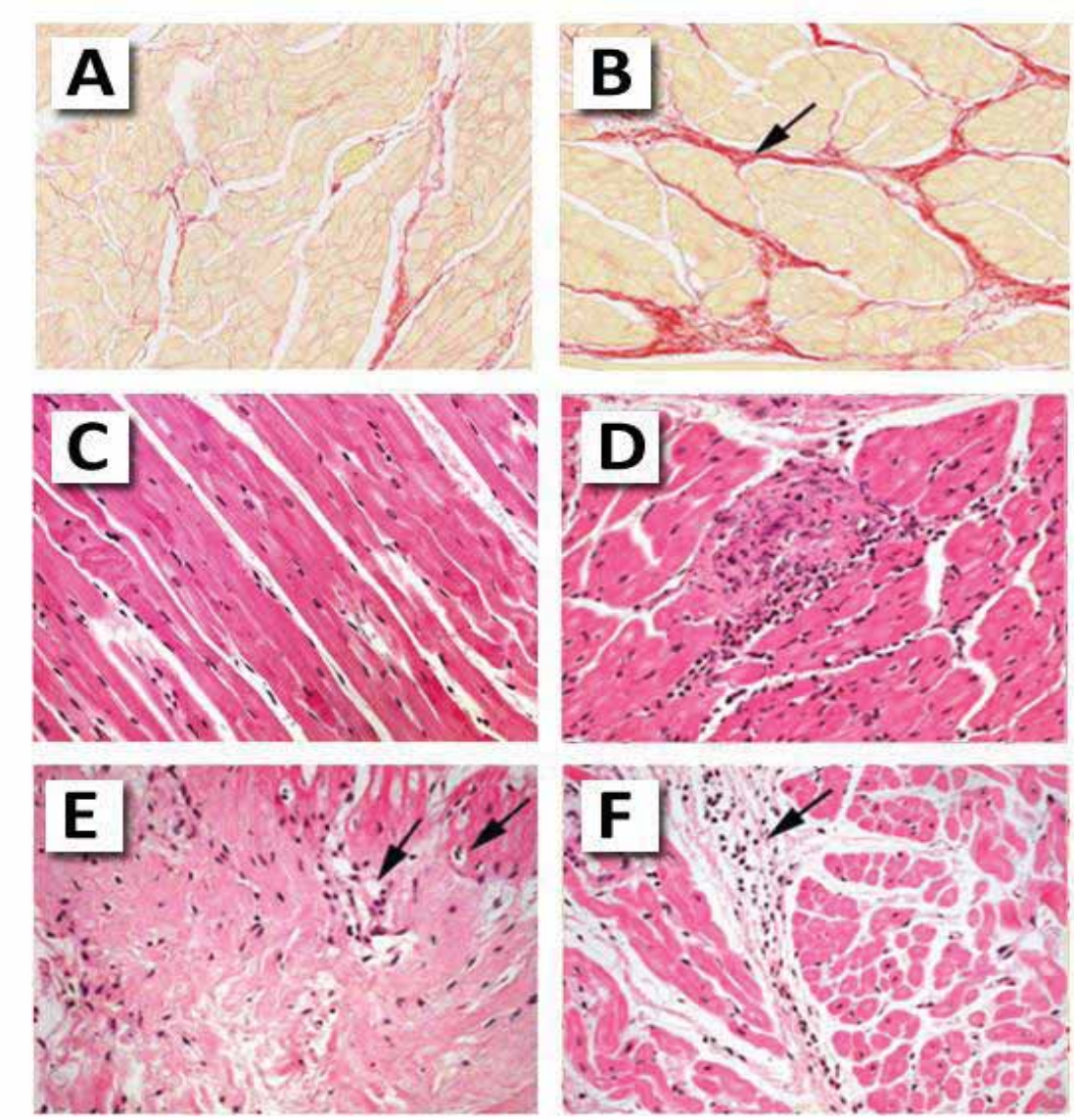
This graph shows the blood pressure profile from baseline through to post nephrectomy and renal clamping in our 1K1C model. A sharp rise in blood pressure is noted immediately after renal clamping to reach a plateau by four weeks.

Figure 2: Atrial and Ventricular Hypertrophy in the Hypertensive Hearts



Cross sectional views of the control (upper left) and hypertensive (upper right) hearts demonstrate the significant ventricular hypertrophy as a result of hypertension. Four chamber views from CMR scans illustrate significant bi-atrial hypertrophy in the hypertensive (lower right) as compared to the control (lower left) heart.

Figure 3: Atrial Histo-pathological Changes



Extensive interstitial fibrosis (red staining) was seen in the hypertensive atrium (B) as compared to control (A) from picrosirius red stains. As compared to control (C), hematoxylin and eosin stains demonstrated atrial myocyte degeneration (D) which was frequently focal and well circumscribed, but (E) occasionally extensive with marked vacuolation and coagulative necrosis (arrow). Extensive lymphocytic infiltration (F) was also evident.

Chapter Three

Short Term Hypertension is Associated with the Development of Atrial Fibrillation Substrate: A Study in an Ovine Hypertensive Model

3.1 Introduction

Due to its high prevalence in the population, hypertension accounts for more atrial fibrillation (AF) than any other risk factors.[327] With the increasing incidence of hypertension and the projected exponential rise in the number of persons with AF, this important synergistic association requires careful attention.[3, 422] Clinical studies have identified a causal relationship between left atrial (LA) size and AF in hypertensive subjects.[327, 347, 423] In addition, the presence of hypertension in AF patients has been shown to result in higher cardiovascular morbidity and mortality.[350] Despite this known association, studies on atrial remodeling due to hypertension remain limited. In particular, whether short duration of hypertension can result in atrial remodeling is not known. By creating experimental hypertension using the “one-kidney, one-clip” (1K1C) model,[424] this study aimed to characterize atrial electrical, structural and functional remodeling in an ovine model of short term hypertension.

3.2 Methods

All procedures were conducted in accordance with the guidelines outlined in the "Position of the American Heart Association on Research Animal Use" adopted on November 11, 1984 by the American Heart Association. This study was approved by the University of Adelaide Animal Ethics Committee, Adelaide, Australia. A total of 16 Merino Cross sheep were studied; 10 with induced hypertension for 7±4 weeks via the 1K1C model and 6 controls.

3.2.1 Animal Preparation and Care

All animals were acclimatized for ≥1week before study and fasted for 24-hours prior to anesthesia for the renal surgeries, cardiac magnetic resonance (CMR) scans and euthanasia electrophysiological study. Intravenous sodium thiopentone (15-20 mg/kg) was used for induction before endo-tracheal intubation, while isoflurane (2-4%) in 100% oxygen was utilized for maintenance. Invasive blood pressure, heart rate, pulse oximetry, end-tidal CO2 and temperature were continuously monitored. Post-operative care included intra-muscular administration of xylazine hydrochloride and penicillin for 3 days.

3.2.2 "One-Kidney, One-Clip" Hypertension

There is no ideal animal model for hypertension given its complex multi-factorial pathogenesis. The 1K1C hypertension has been well studied whereby the primary mechanism for hypertension is due to volume expansion from

sodium and water retention with no involvement of the autonomic or angiotensin system apart from brief initial renin activation during the first one or two weeks after renal clamping.[410, 414, 421, 425] Moreover, given that renovascular hypertension is the most frequent secondary cause of hypertension in humans, the 1K1C model is more physiological than other endocrine, dietary and nephron reduction models.

Unilateral nephrectomy was followed by renal artery clamping using a custom-made Goldblatt-type clamp 3 weeks later. The degree of induced renal artery stenosis was closely regulated to 50-60% with a combination of peri-vascular flow (Transonic Flowmeter TS-401 with 6mm precision flow probe, Transonic Systems Inc., NY, USA) and Doppler velocity (Acuson XP-128, 7MHz probe, Siemens Medical Systems, PA, USA) estimations. The establishment and characterization of this model has been described elsewhere.[424] Blood pressure remained stable from baseline through post unilateral nephrectomy but rose within the first week after renal clamping to reach a plateau by four weeks. Blood pressure was measured in the conscious state via cannulation of exteriorized carotid artery.[411]

3.2.3 Cardiac Functional Assessment

CMR was performed 1-day prior to euthanasia to assess LA and left ventricular volumes and ejection fraction (Siemens Sonata 1.5Tesla MR imaging system, Siemens Medical Solutions, Erlangen, Germany) with slice thickness of 6 mm through the atria and 10 mm through the ventricles without any inter-slice gap. Animals were placed and secured in the dorsal recumbence position for all CMR scans. Mechanical ventilation was maintained to facilitate ECG-gated image acquisition with adequate breath-holding. All analyses were performed offline using Argus software (Leonardo workstation, Siemens Medical Solutions, Erlangen, Germany).

3.2.4 Electrophysiological Study

Bilateral thoracotomy was performed to facilitate open chest electrophysiological studies. Physiological arterial blood gases and body temperature were maintained throughout. Custom designed 128-electrode epicardial plaques with 5mm inter-electrode distance (Figure-1; additional details supplied in Appendix 1, Page 179) were then applied to the RA, LA and traversing the Bachmann's bundle before being attached to a computerized recording system (LabSystem Pro, Bard Electrophysiology, MA, USA). Surface-ECG and overlapping bipolar electrograms were continuously monitored and

stored for off-line analysis. Electrograms were filtered from 30-500Hz, and measured with computer-assisted calipers at a sweep speed of 200mm/s.

3.2.4.1 Atrial Effective Refractory Period

Atrial ERP was measured at twice diastolic threshold at cycle lengths (S1) of 500, 400, 300 and 200ms from six sites (right atrial appendage – RAA, right atrial free wall – RAFW, LAA, LAFW, RA and LA Bachmann's Bundle). Eight basic (S1) stimuli were followed by a premature (S2) stimulus in 10ms decrement. Atrial ERP was defined as the longest S1-S2 interval not resulting in a propagated response. Each measurement was repeated twice and the total averaged. The coefficient of ERP variation (SD/mean x100%) was determined in each atrium to assess ERP heterogeneity.

3.2.4.2 Atrial Conduction

Conduction was assessed during stable S1 pacing from four pre-specified sites (RAA, RAFW, LAA and LAFW). Activation maps were created using semi-automated custom software with manual verification. Local conduction velocity was calculated from the vectors within each triangle of electrodes.[340, 426] Functional conduction was also evaluated from the shortest S2 with a propagated response. The percentage increase in plaque total activation time during S2 was calculated with reference to the corresponding S1 activation maps.

Heterogeneity of Conduction

Conduction heterogeneity was assessed using phase mapping method during S1 pacing and shortest propagated S2.[427] The largest difference in activation time at each site was used to create a phase map and histogram. Absolute conduction phase delay was calculated by subtracting the 5th from the 95th percentile of the phase-difference distribution (P_{5-95}). The conduction heterogeneity index was derived from dividing the absolute phase delay by the median (P_{50}).

3.2.4.3 P-wave Duration

P-wave duration was averaged over 10 consecutive beats as a surrogate marker of inter-atrial conduction time and measured on lead II of the surface-ECG.

3.2.4.4 AF Inducibility and Duration

AF induction was assessed with rapid atrial pacing at the RAA and using premature stimuli. Rapid pacing started at 200ms cycle length and was decreased in 5ms intervals until either AF was initiated or there was loss of 1:1 atrial capture. AF was defined as a rapid irregular atrial rhythm lasting more than 2s. This protocol of induction was repeated ten times if AF duration was <5min and five times if AF duration was between 5-20min. Electrical

cardioversion was applied for episodes lasting >20min or if there was evidence of hemodynamic compromise.

3.2.5 Pathology

Following the conclusion of electrophysiological study, the animal was euthanized using pentobarbital and the heart removed for structural analysis. The LA, LAA, RA and RAA were separated and immersed fixed with 10% formalin and paraffin embedded for subsequent evaluation. Histo-pathological changes were assessed in a blinded protocol. Sections were stained with either haematoxylin & eosin to assess interstitial inflammatory cell infiltration or picrosirius red to demonstrate the extracellular matrix.

3.2.6 Quantification of Collagen Matrix and Inflammatory Infiltrates

To examine the extracellular matrix deposition, 5 randomly selected picrosirius red sections from each individual atrium and appendage of every animal were digitally captured (20 sections/animal). An area of red was selected for its color range and the proportional area of tissue with this range of color was then quantified (Analytical imaging Station, Version 6.0, Ontario, Canada). Likewise, five randomly selected haematoxylin & eosin sections from each individual atrium and appendage of every animal (20 sections per animal) were digitally

captured to quantify inflammatory cell infiltration (Aperio Technologies, Model CS & ImageScope, CA, USA).

3.2.7 Statistical Analysis

All continuous variables are reported as mean±SD and tested for normality utilizing the Shapiro-Wilk test. Normally distributed data were compared using the Student's t-test. Data that were not normally distributed were compared using the Wilcoxon rank-sum tests. Analyses of differential effects of hypertension and over various atrial locations were performed using ANOVA. Repeated measures ANOVA was used when data was analyzed across more than two cycle lengths. Statistical significance was established at $p<0.05$.

3.3 Results

The hypertensive and control groups were matched for size with no significant difference in their weight (53±7 vs. 49±6kg, $p=0.3$) and serum creatinine levels (114±28 vs. 98±20µmol/L, $p=0.2$). The ten 1K1C animals in this study did not exhibit any cardiac complications. In particular, none had clinical evidence of cardiac failure. Blood pressure was significantly higher in the hypertensive sheep (Systolic 176±19 vs. 95±21 mmHg, $p<0.0001$; Diastolic 119±26 vs. 62±14 mmHg, $p<0.0001$).

3.3.1 Anatomical and Functional Remodeling due to Hypertension

The anatomical and functional changes due to hypertension are presented in Table 1. Hypertensive animals demonstrated significantly increased LA volumes with reduced LA function ($p<0.05$). In contrast, left ventricular function was preserved. Cardiac hypertrophy was significant with increased inter-ventricular septal ($p<0.001$) and posterior ventricular wall thickness ($p=0.01$) as well as increased LA ($p=0.001$), RA ($p=0.005$) and left ventricular mass ($p<0.001$).

3.3.2 Electrophysiological Remodeling due to Hypertension

3.3.2.1 Atrial Refractoriness

Atrial ERP was uniformly higher in the hypertensive group (Overall mean ERP averaged across all S1 and sites - RA: 172±23 vs. 157±10ms, LA: 143±14 vs. 127±5ms) at all S1 tested with preservation of physiological rate adaptation (RA: $p=0.0008$, LA: $p<0.0001$; Figure 2A). This was observed at all sites ($p<0.0001$; Figure 2B) without significant difference in ERP heterogeneity at all S1 in the hypertensive versus control group (RA: 17±3 vs. 14±6%, $p=0.4$; LA: 15±1 vs. 18±7%, $p=0.5$; respectively, S1=300ms).

3.3.2.2 Atrial Conduction

Hypertension resulted in slower conduction velocity (Overall mean conduction velocity averaged across all S1 and sites - RA: 0.76±0.02 vs. 0.99±0.10m/s, LA: 0.81±0.05 vs. 1.00±0.04m/s) at all S1 tested (both $p<0.001$, Figure 3A) and at all

sites ($p<0.0001$, Figure 3B). Phase mapping showed an increased absolute range of conduction phase delays (Overall mean P_{5-95} averaged across all S1 and sites – RA: 2.57±0.30 vs. 1.96±0.19ms/mm, $p<0.001$; LA: 2.20±0.20 vs. 1.98±0.09ms/mm, $p=0.009$) and higher conduction heterogeneity index (Overall mean P_{5-95}/P_{50} averaged across all S1 and sites - RA: 1.86±0.08 vs. 1.42±0.06; LA: 1.73±0.09 vs. 1.43±0.07, both $p<0.001$) in the hypertensive group. Differences in both absolute phase delays and conduction heterogeneity index did not change with pacing cycle length (both p=NS). In the hypertensive group, although both absolute phase delays and conduction heterogeneity index appeared higher in the RA as compared to the LA, they did not reach statistical significance (both $p>0.1$).

Activation mapping highlighted increased isochronal crowding in the hypertensive atrium representing slower conduction (Figure 4A-upper panel). Of note, this difference was more marked with shortest propagated S2 (Figure 4A-lower panel) as reflected by the higher mean percentage increase in plaque total activation time over S1 (RA: 63±38 vs. 37±20%, $p=0.03$; LA: 70±25 vs. 43±11%, $p=0.0004$). The corresponding phase histograms demonstrated increased conduction heterogeneity in the hypertensive example during S1 (Figure 4B-upper panel) which was further exaggerated with S2 pacing (Figure 4B-lower panel).

3.3.2.3 P-wave Duration

P-wave duration was significantly increased in the hypertensive sheep (68±3 vs. 56±7ms; p=0.002).

3.3.2.4 AF Inducibility and Duration

The hypertensive group had a greater propensity for the induction of AF (24 vs. 3%; p=0.03). The mean duration of the longest induced AF episode was longer in the hypertensive group (281±624 vs. 11±13s; p=0.04). Two of the hypertensive animals required repeated cardioversion due to prolonged AF of >20min.

3.3.3 Structural Remodeling due to Hypertension

The hypertensive atria demonstrated increased abundance of interstitial inflammatory cells and interstitial fibrosis compared to controls as evidenced by haematoxylin & eosin and picrosirius red staining, respectively. Figure 5 demonstrates picrosirius staining of collagen with a greater content in the hypertensive animals (Mean percentage area: 6.0±2.4 vs. 3.5±1.8%; p<0.0001, Figure 7A). Figure 6 demonstrates much greater inflammatory cell infiltration in each specimen evaluated from hypertensive animals compared to controls (Mean percentage area: 2.0±1.3 vs. 0.4±0.2%; p<0.0001, Figure 7B).

3.4 Discussion

This study presents important new information on the effect of short duration hypertension on the development of the substrate for atrial arrhythmogenesis. In this 1K1C ovine model of hypertension, we observed the following changes within the atria:

1) Anatomical abnormalities characterized by atrial enlargement and dysfunction;
2) Structural abnormalities characterized by increased interstitial fibrosis and inflammatory infiltrates;
3) Marked conduction slowing, increased conduction heterogeneity and prolonged P wave duration;
4) Increased refractoriness at all sites and pacing cycle lengths;
5) Associated with these and perhaps as a consequence, these animals demonstrated an increased propensity to and prolonged duration of AF.

3.4.1 Clinical Association between Hypertension and AF

Hypertension is associated with increased AF risk by 1.5- and 1.4-fold in both men and women.[327] A number of clinical features have been established as predictors of AF development in hypertensive subjects: increasing age, LA enlargement, left ventricular hypertrophy, higher blood pressure level, increased P-wave duration/dispersion and reduced diastolic mitral flow. With a lack of direct electrophysiological evidence on the mechanisms for AF in

hypertension, slowing of atrial conduction can be postulated from increased P-wave duration/dispersion in patients with essential hypertension.

3.4.2 Atrial Substrate for AF

The concept that the atria remodel due to AF is now well established.[96, 102] Central to these observations was the precipitous shortening of the ERP that was proposed to perpetuate arrhythmia maintenance. However, it has become evident that the substrate predisposing to AF may be "different". Li and co-workers in a canine model of congestive heart failure highlighted the importance of interstitial fibrosis and its resultant conduction heterogeneity in sustaining AF.[200] Similar findings were also reported in a canine model of mitral regurgitation.[236] In the clinical setting, prolonged rather than shortened ERP, structural changes and conduction abnormalities have been observed in patients with congestive heart failure, sinus node disease, atrial septal defects and mitral stenosis; all established substrates predisposing to AF.[302-304, 377]

Therefore, our findings in the hypertensive model mirrored closely with previous findings in other disease models.[200, 236, 302-304, 377] This study further highlights the important mechanistic role of atrial fibrosis in atrial remodeling in response to hypertension even at its early stages. Perhaps, the underlying mechanisms to increased AF may share similar patho-physiological etiologies, which are incompletely understood at present.

3.4.3 Atrial Electrophysiological Abnormalities in Hypertension

To date, there is only one study by Kistler and colleagues, which described the atrial electrical and structural effects of chronic hypertension exposure of 5 years duration in an ovine model of prenatal corticosteroid exposure.[299] They uncovered widespread conduction slowing with increased heterogeneity, no change in ERP, increased atrial fibrosis and evidence of cellular myolysis which led to increased AF. However, this study did not determine whether shorter durations of hypertension were adequate to predispose to atrial arrhythmias and there has been concern that this unique model may not be representative of human disease.[351] Therefore our study extends previous observations by demonstrating that even short duration of hypertension resulted in an arrhythmogenic atrial substrate.

3.4.4 Hypertensive Atrial Remodeling: Evidence for a Systemic Process

The finding of bi-atrial abnormalities in this study raises the importance of a systemic process in the development of the arrhythmogenic substrate. There is accumulating evidence to suggest that systemic therapy may alter the development of atrial arrhythmias. Recent large clinical trials involving various angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor antagonists in different clinical subgroups have shown benefits of these agents in reducing AF.[350, 428, 429] Furthermore, an increase in ACE expression and

alterations in angiotensin II receptor expression have been shown in the fibrillating human atria.[430, 431]

Inflammation can be an initiator/perpetuator or a response to underlying AF. Hypertension has been associated with chronic low-level inflammation with recent evidence showing significantly higher C-reactive protein (CRP) levels in hypertensive patients.[432] CRP levels were also associated with AF burden and occurrence.[238, 239] Here, inflammation was observed independent of arrhythmia and may provide a potentially novel target for reversal of atrial remodeling due to hypertension.

3.4.5 Clinical Implications

Our findings strengthen previous work on atrial remodeling due to chronic hypertension by re-affirming the importance of structural abnormalities and the associated conduction changes. However, the important revelation of remodeling within a short duration of hypertension is clinically important. Given the high prevalence of hypertension and recent reports of inadequate blood pressure control in US adults, the AF epidemic may be greater than predicted.[401, 433] This study provides impetus for the early aggressive treatment of hypertension for the prevention of atrial arrhythmias. Finally, our findings of increased atrial fibrosis and inflammation may provide novel targets for more atrial selective therapies in the future.

3.4.6 Study Limitations

Whether the 1K1C model could have contributed to the remodeling seen in our study in addition to the effect of hypertension is not known. However, taken together with the study by Kistler et al in a different model, these changes are most likely representative of hypertension. Whether these changes in 1K1C hypertension can be directly extrapolated to the wide range of clinical hypertensive syndromes is not known.

3.5 Conclusions

Short term hypertension is associated with significant atrial structural and electrical remodeling in the atria. These factors may in part be responsible for the higher frequency of AF in hypertension.

Table 1: Anatomical and Functional Characteristics

	1K1C (n = 10)	Control (n = 6)	P
CMR			
LA EDV (ml)	41.5 ± 6.9	26.7 ± 5.1	0.02
LA ESV (ml)	30.9 ± 3.9	18.0 ± 3.3	0.002
LA EF (%)	25.3 ± 3.3	30.9 ± 2.0	0.04
LV EDV (ml)	86.9 ± 33.8	79.7 ± 14.5	0.1
LV ESV (ml)	40.8 ± 19.0	41.7 ± 12.7	0.2
LV EF (%)	42.9 ± 8.8	44.3 ± 5.4	0.6
Pathology			
LA wt (g)	35 ± 6	21 ± 4	0.001
RA wt (g)	22 ± 4	16 ± 3	0.005
IVS wt (g)	65 ± 8	46 ± 6	0.001
LV wt (g)	157 ± 20	97 ± 8	< 0.001
LV wt/Body wt (g/kg)	2.8 ± 0.4	2.1 ± 0.3	0.008
IVS (mm)	18.1 ± 2.1	13.0 ± 0.7	< 0.001
PW (mm)	14.3 ± 2.1	10.8 ± 0.9	0.01

EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction; IVS, inter-ventricular septum; LA, left atrial; LV, left ventricular; PW, posterior left ventricular wall; RA, right atrial; wt, weight.

Figure 1: Epicardial Plaque Design

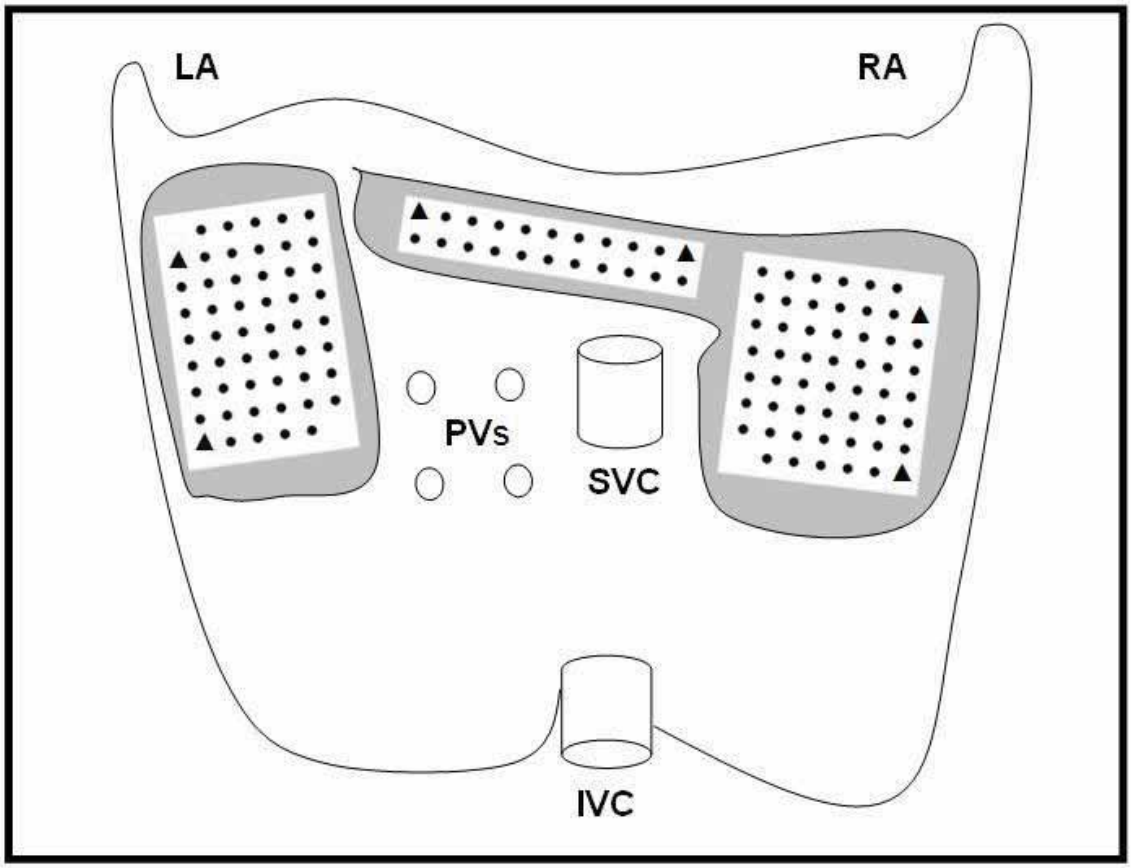
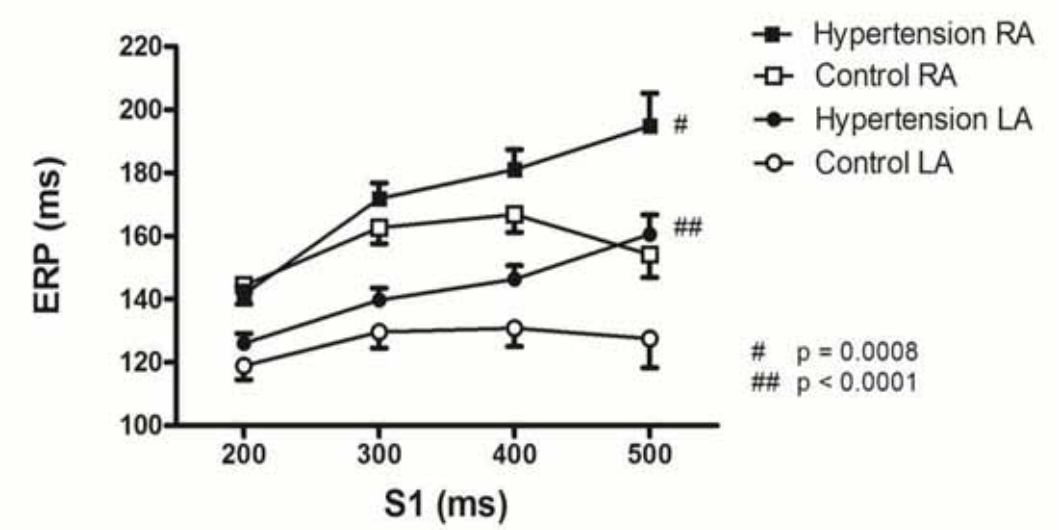


Illustration of plaque design showing a total of 128 electrodes covering the left atrium, right atrium and Bachmann's bundle. Triangular points represent pre-specified pacing sites.

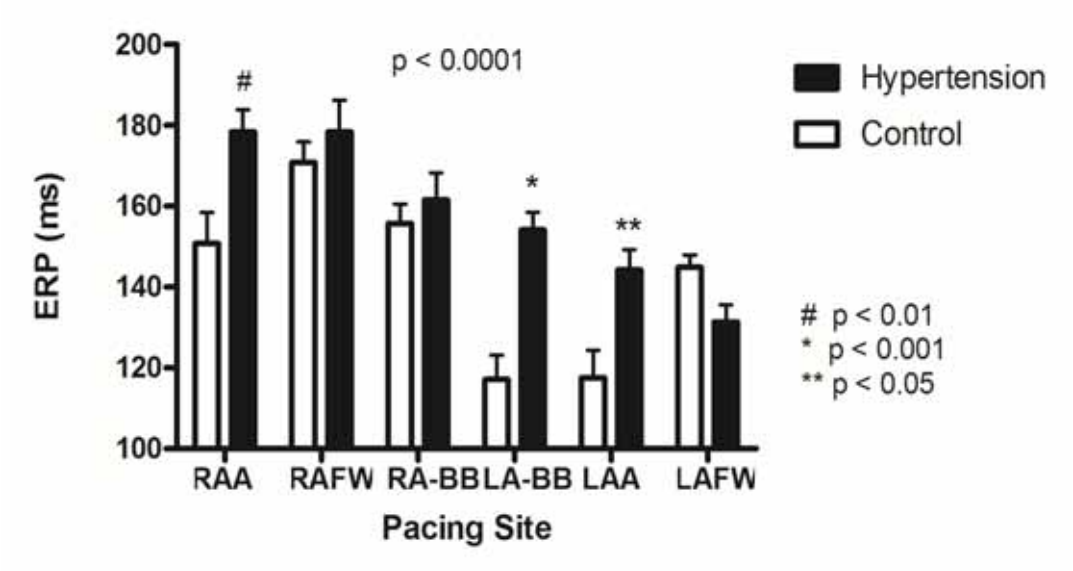
Figure 2: Atrial ERP at different pacing cycle lengths and sites

A Mean ERP at different pacing cycle lengths



ERP (mean±SEM) was uniformly higher in the hypertensive group at all pacing cycle lengths compared to controls. This graph utilized pooled data from all pacing sites of the respective atrium.

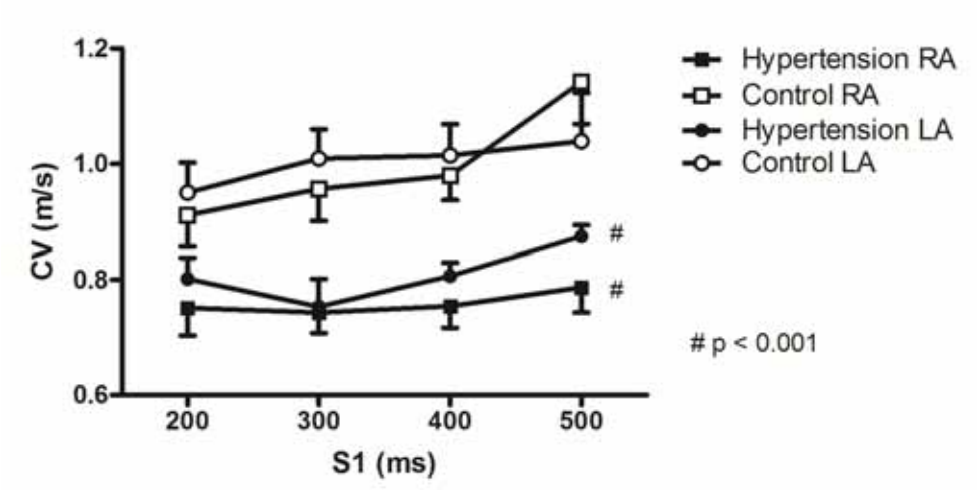
B Mean ERP at different pacing sites (S1=300ms)



Similarly, ERP (mean±SEM) was higher in the hypertensive group when analyzed at different atrial sites. This graph illustrates the difference between the two groups during pacing cycle length of 300ms.

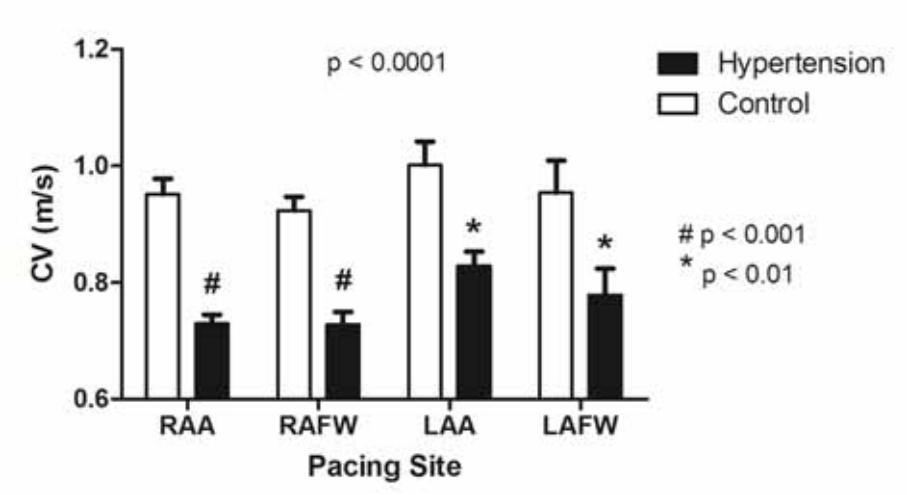
Figure 3: Conduction velocity and conduction heterogeneity

A Mean CV at different pacing cycle lengths



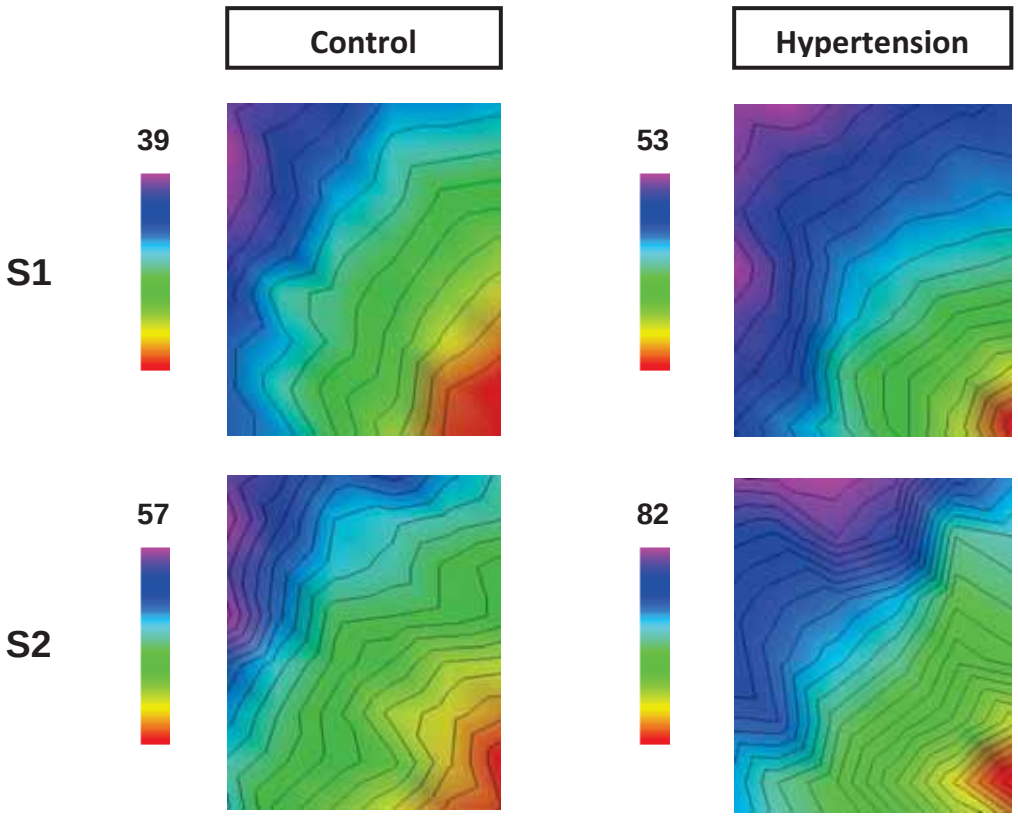
Hypertension resulted in slower conduction velocity (mean±SEM) at all pacing cycle lengths. This graph utilized pooled data from all pacing sites of the respective atrium.

B Mean CV at different pacing sites (S1=300ms)



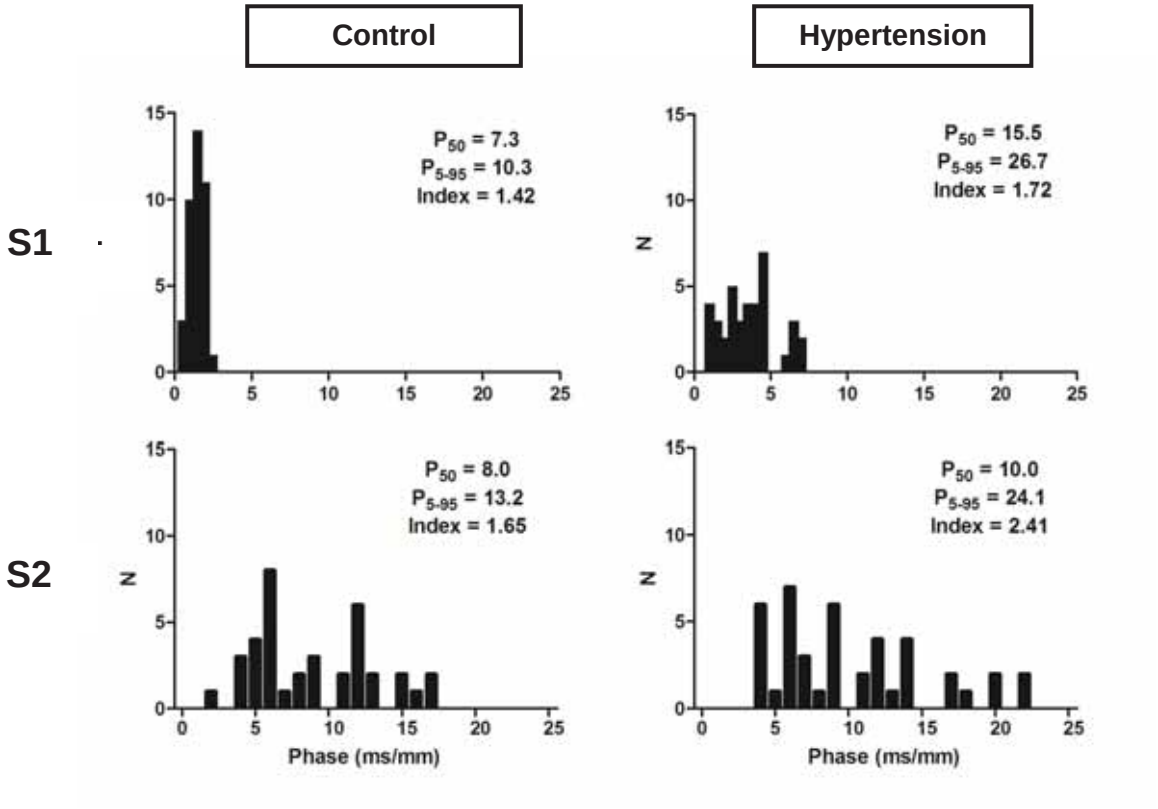
The same difference in conduction velocity is seen during pacing from different sites. This graph shows conduction velocity in the two groups with S1 at 300ms.

Figure 4A: Representative Activation Maps



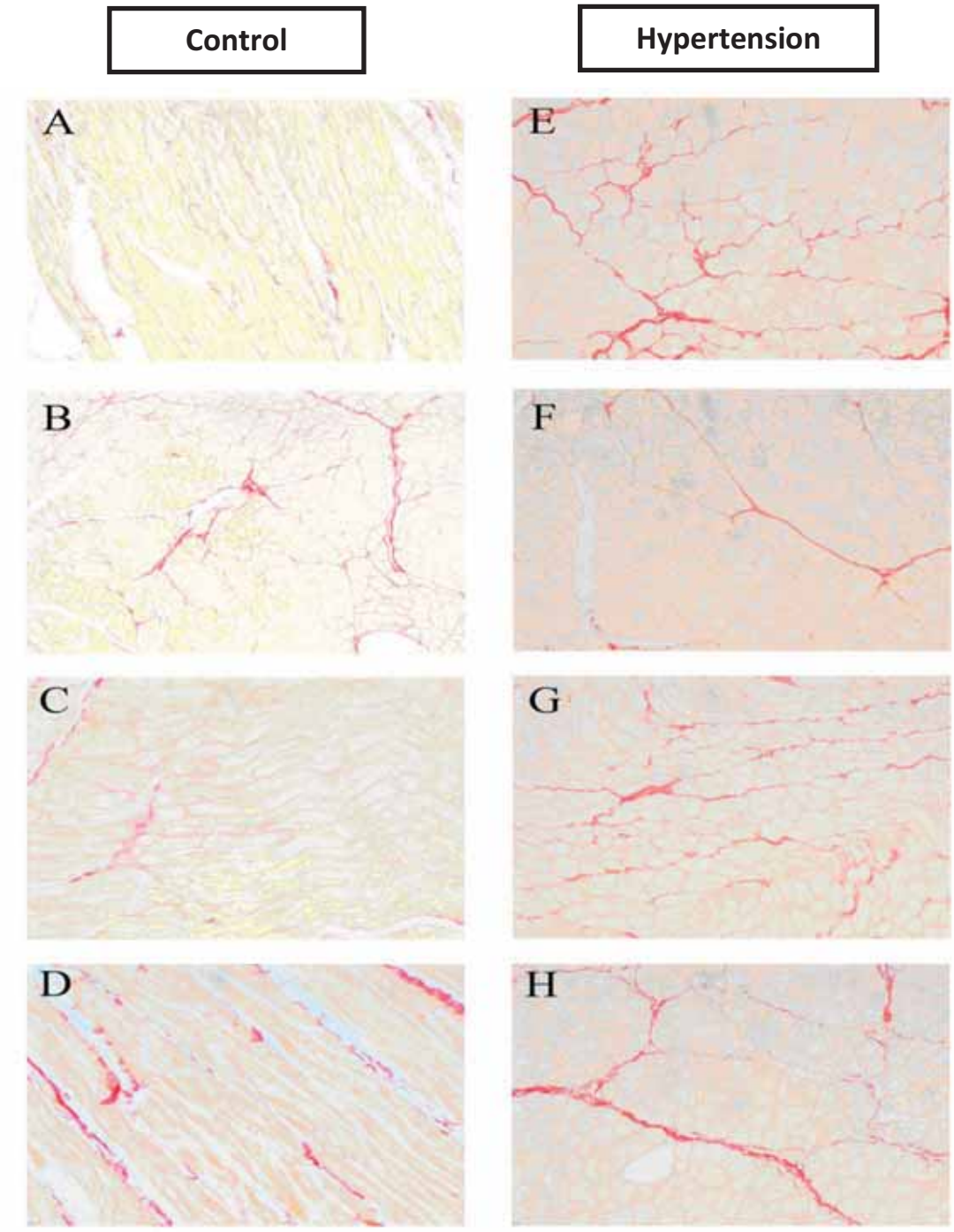
Representative activation maps from control (left panel) and hypertensive (right panel) atria during S1 (300ms, upper panel) and S2 (lower panel) pacing from the LAA. Isochrones have been constructed at 3ms intervals. Within the short duration of hypertension, isochronal crowding is evidenced in the hypertensive atrium which is indicative of slower conduction. This difference was further exaggerated during S2 pacing.

Figure 4B: Corresponding Phase Histograms



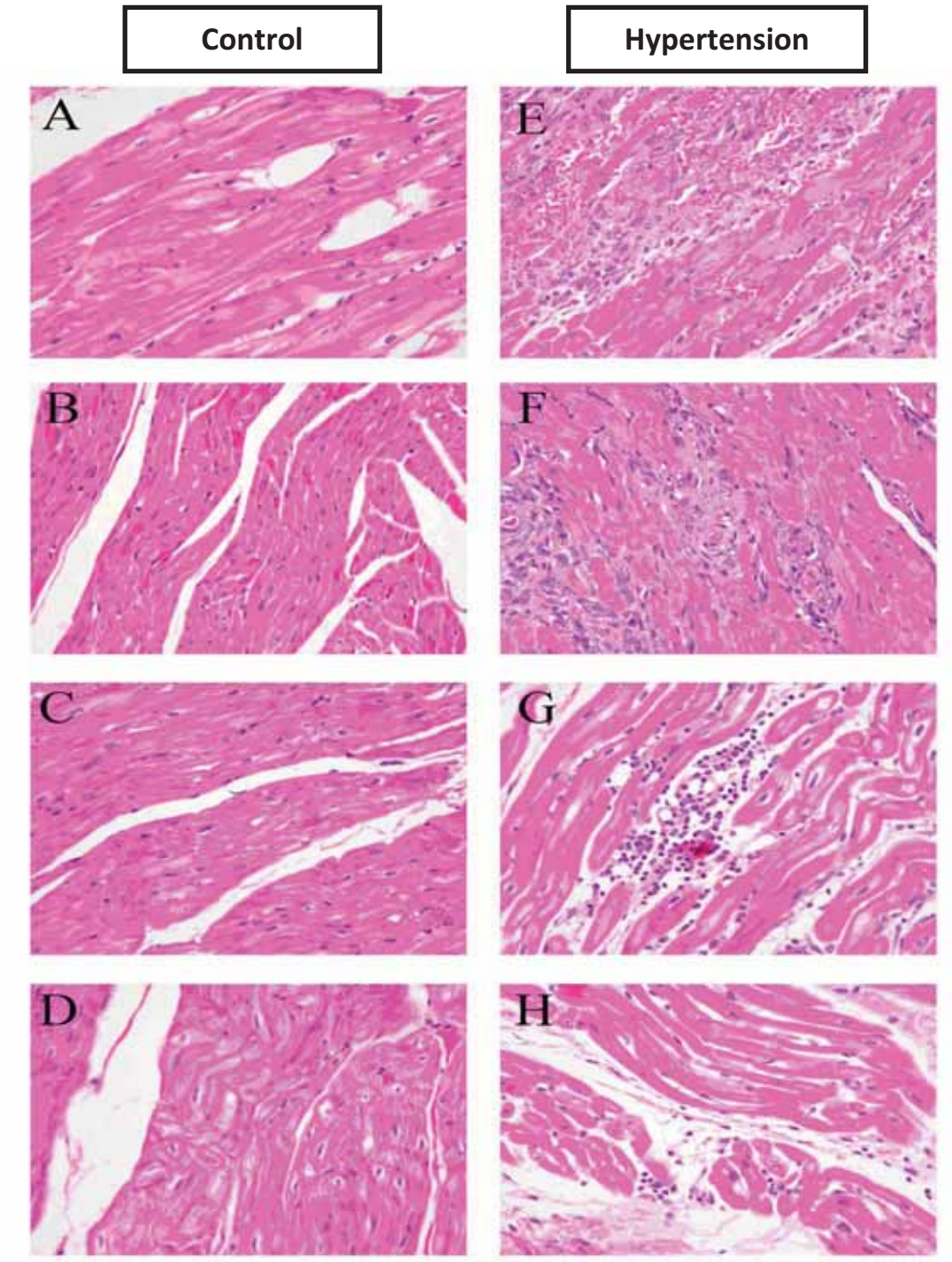
Corresponding phase histograms from activation maps shown in Figure 4A during S1 (300ms) and S2 pacing from the LAA. Conduction heterogeneity index during S1 was higher in the hypertensive vs. control atria (upper panel). Likewise, during S2 pacing, a greater increase in heterogeneity was seen in the hypertensive example.

Figure 5: Representative Picrosirius Red Sections



In control LA (A), LAA (B), RA (C) and RAA (D), little collagen (red staining) was present within the interstitium, while extensive interstitial fibrosis was noted in hypertensive LA (E), LAA (F), RA (G) and RAA (H). [Magnification x350]

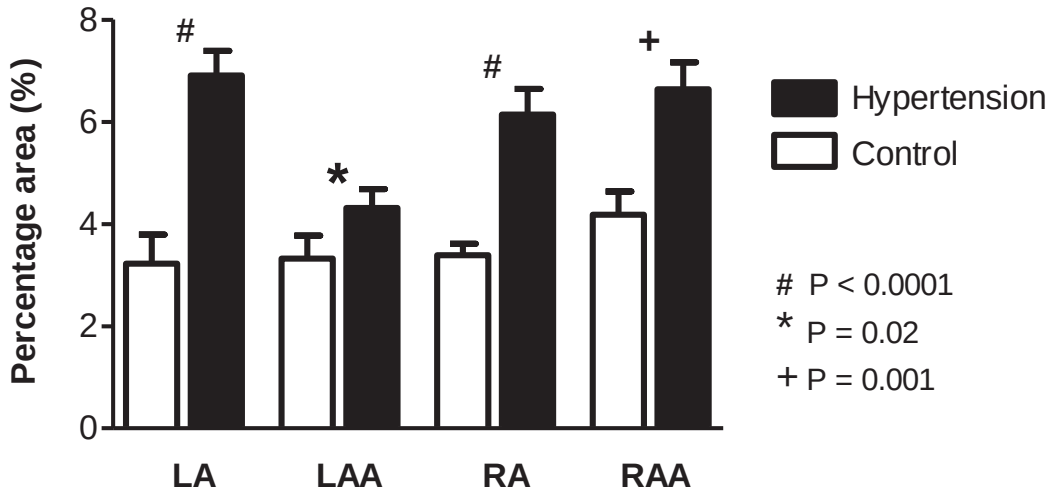
Figure 6: Representative H & E Sections



In control LA (A), LAA (B), RA (C) and RAA (D), occasional inflammatory cells was present within the interstitium versus extensive interstitial inflammatory cells in hypertensive LA (E), LAA (F), RA (G) and RAA (H). [Magnification x350]

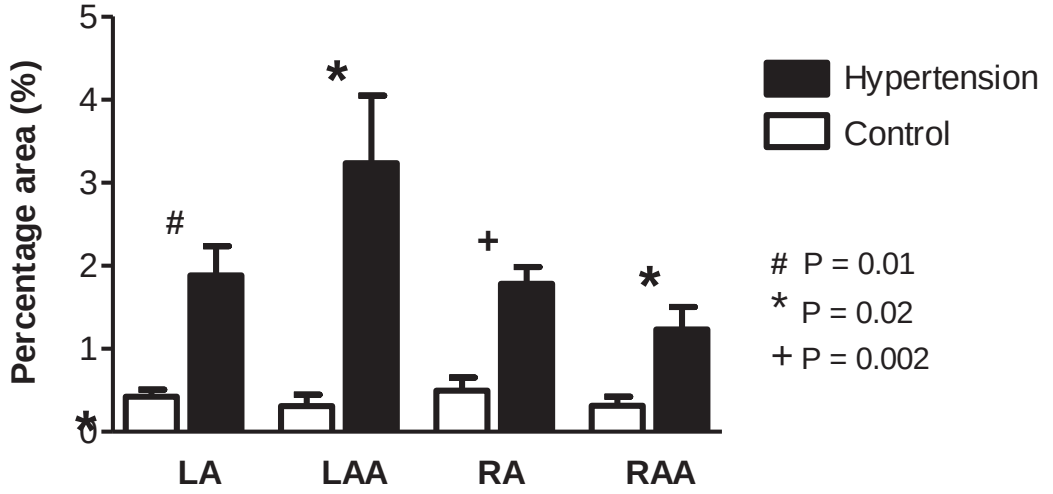
Figure 7: Histological Analyses

A Quantification of Collagenous Matrix



Significantly greater collagen content (mean±SEM) was seen in the hypertensive animals as compared to controls in LA, LAA, RA and RAA.

B Quantification of Inflammatory Infiltrates



Likewise, significantly greater percentage area of inflammatory infiltrates (mean±SEM) was seen in the hypertensive LA, LAA, RA and RAA as compared to the controls.

Chapter Four

Hypertension and Atrial Fibrillation: Evidence of Progressive Atrial Remodeling with Electro-Structural Correlate in a Conscious Chronically Instrumented Ovine Model

4.1 Introduction

The important synergistic association of hypertension as a risk factor for atrial fibrillation (AF) requires further evaluation given the increasing burden of both conditions.[3, 422] Current understanding on the mechanistic link remains limited despite the profound impact of hypertension on the clinical course of AF.[350, 434] Recently, studies in large animal models have provided some insights on atrial remodeling in hypertension.[299, 435] However, the time course of these changes has not been studied.

We hypothesize that the development of hypertension is associated with progressive atrial remodeling leading to a greater propensity to AF. By creating experimental hypertension using the "one kidney-one clip" (1K1C) model, this study aimed to characterize the time course, extent and electro-structural correlation of atrial remodeling in the conscious chronic ovine hypertensive model.

4.2 Methods

Thirty-two Merino Cross wethers were studied. All procedures were conducted in accordance with the guidelines outlined in the “Position of the American Heart Association on Research Animal Use” adopted on November 11, 1984 by the American Heart Association. This study was approved by the Animal Ethics Committee of the University of Adelaide, Adelaide, Australia.

4.2.1 Study Protocol

All animals (n=32) were acclimatized for at least one week prior to commencement of their respective study protocol (Figure 1). 12 animals underwent sequential closed chest electrophysiological evaluations while 20 underwent terminal open chest evaluations to allow for electro-structural correlation sans the influence of chronically implanted plaque on histological parameters. Hypertension was induced with the 1K1C model (n=6 in the closed chest study cohort and n=15 in the open chest study cohort) as described below. Animals in the open chest study arm were randomly sacrificed at different stages of hypertension: baseline, 5, 10 and 15 weeks for electrical and structural analysis.

4.2.2 General Anesthesia

General anesthesia was utilized for nephrectomy, renal artery clamping, cardiac magnetic resonance imaging (CMR) and bilateral thoracotomy for epicardial plaque implantation. Sodium thiopental was used for induction to facilitate endotracheal intubation and isoflurane for maintenance. Invasive blood pressure, heart rate, end-tidal CO_2 and temperature were continuously monitored. Specifically, mechanical ventilation was maintained during CMR scans to facilitate ECG-gated image acquisition with adequate breath-holding.

4.2.3 "One Kidney-One Clip" Hypertension

Hypertension in this study was induced using the 1K1C model as characterized elsewhere.[436] In brief, unilateral nephrectomy was followed by clamping of the remaining renal artery (~30% stenosis) using custom made Goldblatt-type clamp three weeks later. Renal function was monitored by serum creatinine levels.

4.2.4 Cardiac Functional Assessment

CMR was utilized to assess both left atrial and ventricular volumes/ejection fraction during hypertension (Siemens Sonata 1.5 Tesla MR imaging system, Siemens Medical Solutions, Erlangen, Germany) with slice thickness of 6 mm through the atria and 10 mm through the ventricles without any inter-slice gap. Animals were placed and secured in the dorsal recumbence position during

CMR scans. All analyses were performed offline using Argus software (Leonardo workstation, Siemens Medical Solutions).

4.2.5 Electrophysiological Study

Bilateral thoracotomy was performed to allow implantation of a custom designed 128-electrode epicardial plaques over the RA, LA and traversing the Bachmann's bundle in 12 animals (6 hypertensive and 6 controls) for sequential closed chest studies in the conscious animals.[435] The wirings and connectors were passed through the right inter-costal space and tunneled subcutaneously to exit dorsally where they were kept secure in a pouch. For open chest terminal electrophysiological evaluations, the epicardial plaques were applied in the same manner without tunneling of the wirings and connectors. During electrophysiological studies, the connectors were attached to a computerized electrophysiology recording system (LabSystem Pro, BARD Electrophysiology, Lowell, MA). Surface-ECG and sequential overlapping bipolar electrograms were continuously monitored and stored for off-line analysis. The following parameters were determined:

4.2.5.1 Atrial ERP

Atrial ERP was measured at twice diastolic threshold at 500, 400, 300 and 200ms from six sites (right atrial appendage, RAA; RA free wall, RAFW; RA

Bachmann's Bundle, RA-BB; LAA, LAFW, and LA-BB). Eight basic (S1) stimuli were followed by a premature (S2) stimulus in 10ms decrement. Atrial ERP was defined as the longest non-propagated S1-S2. Coefficient of ERP variation (standard deviation/mean x 100%) was determined to assess ERP heterogeneity.

4.2.5.2 Atrial Conduction

Conduction was assessed during stable S1 pacing at 500, 400, 300 and 200ms from the RAA, RAFW, LAA and LAFW. Activation maps of the atria were created using semi-automated custom software and verified manually. Isochronal lines of 3ms increment were superimposed to further illustrate conduction patterns. Conduction velocity was calculated from the local vectors within each triangle of electrodes.[340, 426]

Heterogeneity of Conduction

Conduction heterogeneity was assessed using established phase mapping method during S1 pacing. Absolute conduction phase delay was calculated by subtracting the 5^{th} from the 95^{th} percentile of the phase-difference distribution (P_{5-95}). Conduction heterogeneity index was derived from dividing P_{5-95} by the median (P_{50}).

4.2.5.3 P-wave Duration

P-wave duration was averaged over ten consecutive beats as a surrogate marker of inter-atrial conduction time and measured on lead II of the surface-ECG.

4.2.5.4 AF Inducibility and Duration

AF inducibility was assessed using a standardized protocol of burst pacing in the RAA and during programmed extra-stimuli. Pacing started at 200ms and was decreased in 5ms intervals until either AF was initiated or there was loss of 1:1 capture. A fixed rate of decrement was used at 5ms per 3s to ensure consistency. AF was defined as a rapid irregular atrial rhythm lasting >2s. This protocol of induction was repeated ten times. Mean AF duration was obtained from the average of all induced AF episodes.

4.2.5.5 Electrogram Fractionation during AF

Complex fractionated atrial electrograms during AF were analyzed using custom software. The algorithm was based on validated algorithm with fractionation expressed as CFE-mean.[437] In brief, it detects and annotates deflections on the electrograms determined by the "peak-to-peak sensitivity" (0.05-0.1mV), amplitude duration (10ms) and refractory period (30ms). To ensure accurate characterization, only episodes lasting ≥8s were included.

4.2.6 Structural Analysis

At the end of the study protocol, animals were euthanized using pentobarbital and the hearts removed for structural analysis. The LA, LAA, RA and RAA were separated and immersed fixed with 10% formalin and paraffin embedded for subsequent light microscopic evaluation. Histopathological changes were assessed in a masked protocol. Sections were stained with picrosirius red to demonstrate the extracellular matrix or haematoxylin & eosin to assess inflammatory cell infiltration. Quantification of collagen matrix and inflammatory infiltrates were performed digitally involving five random sections from each atrium/appendage of every animal (20 sections/animal) as previously described.[435]

4.2.7 Statistical Analysis

All continuous variables are reported as mean±standard deviation and assessed for normality utilizing the Shapiro-Wilk test. ANOVA was used for comparisons of structural and functional data in different groups at different time points. A linear mixed effects model was used to compare changes over time of electrophysiological data between the two groups with hypertension, time and their interaction modeled as fixed effects. Correlation was analyzed using

Pearson or Spearman correlation coefficient. Statistical significance was established at $p<0.05$.

4.3 Results

In the hypertensive group, a progressive increase in arterial blood pressure was observed from baseline through to 5, 10 and 15 weeks of hypertension (Figure 2, $p<0.001$ for the increasing trend from baseline). There was no significant difference in the corresponding serum creatinine levels (104±30 vs. 93±16 vs. 96±39 vs. 97±24mmol/L; p=NS).

4.3.1 Anatomical and Functional Remodeling

Cardiac hypertrophy was evident during the development of hypertension with bi-atrial enlargement and increased left ventricular dimensions (Table 1). CMR assessment demonstrated an increasing LA volume with significant decline in LA ejection fraction from 5 weeks of hypertension. In contrast, left ventricular size and function remained unchanged representing compensated left ventricular hypertrophy.

4.3.2 Sequential Closed Chest Electrophysiological Studies: Progressive Electrical Remodeling

4.3.2.1 Atrial Refractoriness

ERP were uniformly higher at all time points during hypertension as compared to controls in both atria (Table 2); this being significant at each site and cycle length tested (both $p<0.001$). However, in both groups, mean ERP did not vary with time over the course of 15 weeks (p=NS). Indeed, elevation of ERP occurred very early on within the first five weeks of hypertension exposure. ERP heterogeneity was similar in the hypertensive versus control group and did not change over time (10±6 vs. 10±7% at baseline, 12±5 vs. 15±9% at 5 weeks, 9±3 vs. 16±7% at 10 weeks and 10±7 vs. 11±8% at 15 weeks respectively; p=NS).

4.3.2.2 Atrial Conduction

The development of hypertension resulted in progressive and significant atrial conduction slowing which was more pronounced from 10 weeks onwards while mean conduction velocity of the control group showed no significant change with time (Table 2). In addition, a paralleled increase in conduction heterogeneity index and the absolute range of conduction phase delays were seen in the hypertensive group. Representative sequential color activation maps shown in Figure 3A demonstrated progressive conduction slowing (isochronal crowding) at different stages of hypertension. Similarly, the corresponding

phase histograms showed the increasing phase delays accounting for increasing conduction heterogeneity at different stages of hypertension (Figure 3B).

4.3.2.3 P-wave Duration

P-wave duration increased progressively during hypertension (58±5ms at baseline, 65±4ms at 5 weeks, 69±6ms at 10 weeks and 72±3ms at 15 weeks, $p<0.001$).

4.3.2.4 AF Inducibility

The propensity for the induction of AF was apparent early during hypertension: from 2±4% at baseline, 20±16% at 5 weeks, 25±18% at 10 weeks & 20±16% at 15 weeks as compared to 0.3±0.7%, 4±5%, 2±4% & 5±6% in the control group at the respective time points ($p=0.003$). In addition, while the mean AF duration was consistently longer at all time points, it became more prolonged only with longer duration of hypertension (3±4 vs. 1±2s at baseline, 11±10 vs. 2±3s at 5 weeks, 14±9 vs. 4±4s at 10 weeks & 30±38 vs. 2±3s at 15 weeks; $p=0.04$).

4.3.2.5 Atrial Fractionation during AF

Most AF episodes from control animals were less than 8 seconds duration and therefore could not be analyzed. A total of 1947 8-second induced AF recordings at different stages of hypertension were analyzed. A progressive trend at 5, 10

and 15 weeks of hypertension in increasing fractionation (CFE-mean: 150±24 vs. 142±25 vs. 137±24ms, respectively; p<0.001) and increasing proportion of CFE-mean <120ms (14.4% vs. 21.5% vs. 26.8%, respectively; p<0.001) was observed. This was concurrent with the increasing AF inducibility and longer AF episodes as described above.

4.3.3 Structural Remodeling

The hypertensive atria demonstrated increased interstitial fibrosis compared to controls. Figure 4A demonstrates the representative changes of increasing interstitial fibrosis in the hypertensive atria showing greater content of collagen highlighted by picrosirius staining with progressive hypertension. Quantification of collagen deposition showed a progressive increase in fibrosis with greater changes evident from longer duration of hypertension (Figure 4C). There was also a significant increase in inflammatory infiltrates from 5 weeks of hypertension (Figures 4B & D).

4.3.4 Open Chest Electrophysiological Studies: Electro-Structural Correlation

Figure 5 demonstrates the correlation between the structural changes of fibrosis/inflammation and the electrophysiological parameters. Increased atrial fibrosis was significantly correlated with decreased conduction velocity,

increased conduction heterogeneity index, longer induced AF episodes and a trend to greater AF inducibility (Figures 5A-D). Increased atrial inflammation was found to correlate only with higher conduction heterogeneity index and greater AF inducibility (Figures 5E-H).

4.4 Discussion

This study characterized the progressive atrial remodeling due to hypertension in a chronic conscious ovine model. Over a 15 week period, progressive anatomical, functional, electrophysiological and structural changes were seen within the hypertensive atria. However, these changes occurred at different time domains (Figure 6).

Remodeling in early hypertension (≤5 weeks) which was associated with:

- Significant atrial dilatation and hypertrophy;
- Functional impairment with reduced atrial ejection fraction;
- Higher refractoriness;
- Increased inflammatory infiltrates.

As a result of these early changes there was an increased inducibility of AF.

Remodeling with prolonged duration of hypertension (≥10 weeks) was associated with:

- Increased atrial interstitial fibrosis;
- Progressive conduction slowing with increased heterogeneity.

These progressive changes resulted in more sustained AF and greater fractionation of the atria during AF.

The conduction changes seen with marked interstitial fibrosis in the hypertensive atria are similar to previous findings in other experimental models or clinical conditions with chronic atrial remodeling.[169, 200, 236, 302-304, 377] However, the current study demonstrates the important progressive nature of atrial remodeling with longer duration of hypertension. In particular, the later development of atrial fibrosis and the electrophysiological consequence of more sustained AF highlighted its important mechanistic role in hypertension. This was further validated with significant correlation shown between atrial fibrosis and both atrial conduction abnormalities and duration of AF episodes. The finding of increased atrial inflammation evident from early hypertension is of significance. Increased inflammation has been reported in chronic atrial dilatation and sterile pericarditis models but not in the commonly studied atrial or ventricular tachypacing models.[236, 237] Its variable presence in different AF substrates and the significant correlation seen with increased conduction heterogeneity and AF inducibility in this study would provide for an attractive alternate treatment target in the remodeled hypertensive atria. In particular,

inflammation is known to contribute to both the initiation and perpetuation of AF.[232]

4.4.1 Time Course of Atrial Remodeling in Hypertension

The remodeling cascade described in this study resembles the time course of atrial changes in tachycardia induced AF models. Structural remodeling has been found to occur at a different time domain as compared to electrical remodeling. Electrical remodeling occurs within the first few days of AF and goes "hand in hand" with contractile remodeling.[102, 211] In contrast, structural remodeling occurs progressively but much later from weeks to months of AF and may contribute to the development of persistent AF.[174, 226] Importantly, reverse electrical remodeling has been shown to occur within a few days while recovery from structural remodeling required a much longer time period.[102, 211, 227] Of note, similar sequence of remodeling has also been described during the development and reversal of congestive heart failure in the canine model.[438, 439] Therefore, this characterization of the time course of atrial changes in hypertension presents a window for intervention before remodeling progresses to an unfavorable structural milieu capable of maintaining AF. This translates to a threshold in a hypertensive individual whereby atrial fibrosis will ensue to reach a point of no return in relation to its arrhythmogenicity.

4.4.2 Compounding Detrimental Effects of Hypertension and AF

The progressive nature of AF was first demonstrated elegantly in animal models in the 1990s.[96, 102] While various studies in lone AF patients have demonstrated a low risk of progression to chronic AF, the presence of co-morbidities in patients with paroxysmal AF was associated with greater predisposition to develop chronic AF.[440] In addition, both progression of AF in hypertensive patients and the presence of hypertension in AF patients have been found to result in more AF associated morbidities and mortality.[350, 434] While treatments with various anti-hypertensive agents have been shown to prevent AF, we do not yet have data on the blood pressure target to aim for.

4.4.3 Upstream Targeting as Primary AF Prevention?

Recent work has demonstrated that hypertension is a strong and independent predictor of incident AF even at systolic blood pressure in the prehypertension levels (<140 mmHg).[441] This finding was not surprising given that prehypertension has been found to be associated with increased risk of cardiovascular disease.[442] Specifically, patients with prehypertension have greater left atrial stress due to impaired left ventricular diastolic function resulting in longer P wave duration, increased P wave dispersion and larger left atrial dimension which may contribute to their increased risk of developing AF. With the high prevalence of prehypertension in U.S. adults estimated at more

than 30%, targeting of prehypertension may prove to be an attractive option in reducing the escalating AF epidemic since it is highly likely that there is a continuum of atrial changes in the remodeling cascade even during the prehypertension stage.[443]

4.4.4 Clinical Significance

Our findings highlighted the important role of atrial fibrosis and its resultant conduction abnormalities with more prolonged duration of hypertension leading to more sustained AF. The revelation of progressive remodeling with longer hypertension exposure is clinically important. Given the high prevalence of prehypertension and hypertension, together with the inadequacy of blood pressure control in US adults, the AF epidemic may be greater than what physicians and health authorities have predicted.[433] This study provides impetus for the early aggressive treatment of hypertension in arresting the cascade of atrial changes to prevent atrial arrhythmias. Perhaps, future studies may provide further direction in upstream targeting of hypertension as primary AF prevention together with absolute blood pressure target. Finally, this study highlighted the importance of atrial fibrosis and inflammation in hypertension which may be novel targets for more atrial selective therapies in the future.

4.4.5 Study Limitations

We utilized an established experimental hypertensive model with physiological high blood pressure levels. However, it remains uncertain whether we can directly extrapolate our findings to a wide range of human hypertensive syndromes. In particular, it is not known whether this model of hypertension causes activation of any specific inflammatory or pro-fibrotic mediators. The strength of repeated electrophysiology studies in the conscious animals outweighs once-off open chest studies in different anesthetized animals even though the effect of a chronically implanted epicardial plaque on electrical remodeling remains unknown. We have controlled for this limitation by comparing our electrophysiological data to a control group. However, our control group did not undergo sham renal surgeries.

4.5 Conclusions

This study presents a detailed time course of the early and progressive atrial remodeling due to hypertension. There are progressive structural changes associated with conduction abnormalities which resulted in greater inducibility and duration of AF. These changes demonstrate significant electro-structural correlation.

Table 1: Anatomical and Functional Characteristics

	Baseline (n=5)	5 Weeks (n=5)	10 Weeks (n=5)	15 Weeks (n=5)	P
Pathology					
LA wt (g)	21.4±4.3	27.2±7.6	34.9±3.8	29.9±5.2	0.003
RA wt (g)	15.7±3.4	18.7±5.1	20.6±4.2	19.5±2.5	0.003
IVS wt (g)	42.4±1.2	46.8±3.6	51.0±7.5	56.5±5.4	0.03
LV wt (g)	98.1±7.9	101.4±10.5	112.5±20.7	118.2±14.7	0.02
IVS (mm)	12.1±0.7	16.2±0.8	15.8±0.3	16.5±1.4	0.005
PW (mm)	9.2±1.0	10.7±0.8	12.5±0.5	11.9±1.3	0.002
CMR					
LA EDV (ml)	24.9±3.3	35.6±3.9	38.9±11.6	38.9±5.5	0.02
LA EF (%)	33.4±2.9	30.9±4.2	29.3±1.6	28.3±0.8	<0.05
LV EDV (ml)	84.6±5.6	97.0±16.7	99.5±20.0	96.3±6.8	NS
LV EF (%)	46.3±3.8	43.7±6.6	42.6±4.2	42.9±5.1	NS

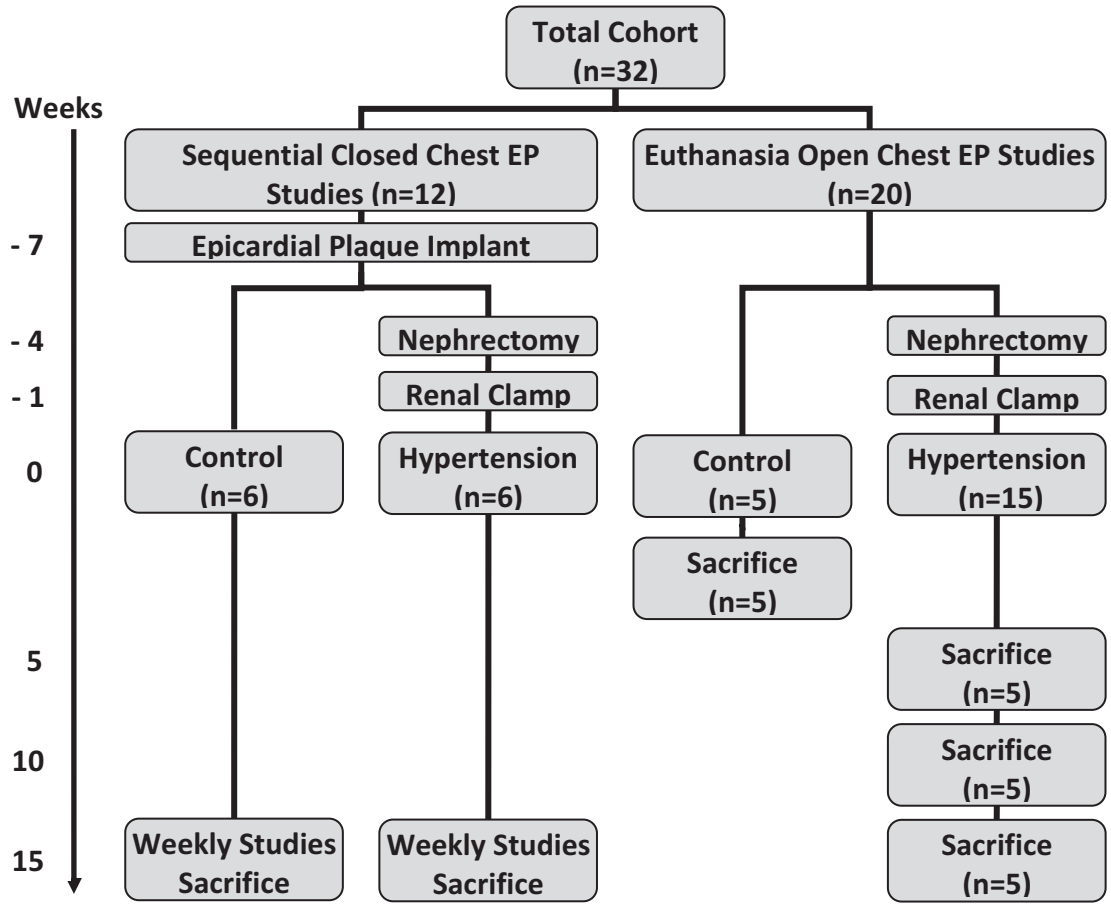
LA, left atrial; RA, right atrial; IVS, inter-ventricular septum; LV, left ventricular; PW, posterior left ventricular wall; EDV, end diastolic volume; EF, ejection fraction; wt, weight.

Table 2: Sequential Closed Chest Electrophysiological Parameters

	Baseline	5 Week	10 Week	15 Week	$p^{\#}$	$p^{\wedge}$
ERP, S1=300ms (ms)						
HT, RA	174±11	206±15	198±26	191±12		
Control, RA	174±9	164±16	181±21	172±15	0.03	NS
HT, LA	130±14	142±16	141±7	147±18		
Control, LA	128±13	117±16	131±16	126±3	0.04	NS
CV (m/s)						
HT	0.92±0.08	0.89±0.12	0.85±0.13	0.80±0.13		
Control	0.98±0.20	1.03±0.12	1.06±0.11	1.06±0.15	0.03	0.001
P_{5-95} (ms/mm)						
HT	1.45±0.32	1.85±0.80	2.10±0.68	2.72±0.81	0.08	<0.001
Control	1.40±0.42	1.33±0.26	1.41±0.37	1.58±0.98		
CHI: P_{5-95}/P_{50}						
HT	1.08±0.20	1.31±0.32	1.72±0.49	1.87±0.51	<0.001	<0.001
Control	1.12±0.29	1.15±0.21	1.20±0.23	1.20±0.26		

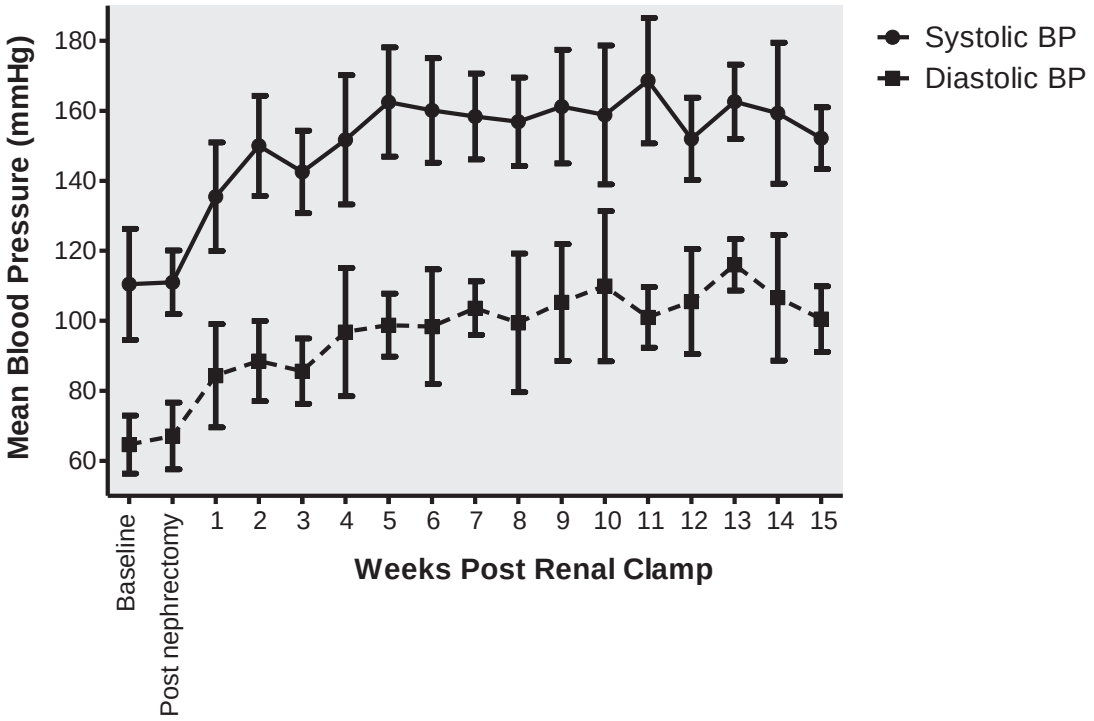
$p^{\#}$, p value for effect of hypertension; $p^{\wedge}$, p value for effect of hypertension and time; RA, right atrial; LA, left atrial; ERP, effective refractory period; CV, conduction velocity; P_{5-95}, absolute conduction phase delay; CHI, conduction heterogeneity index.

Figure 1: Study Design



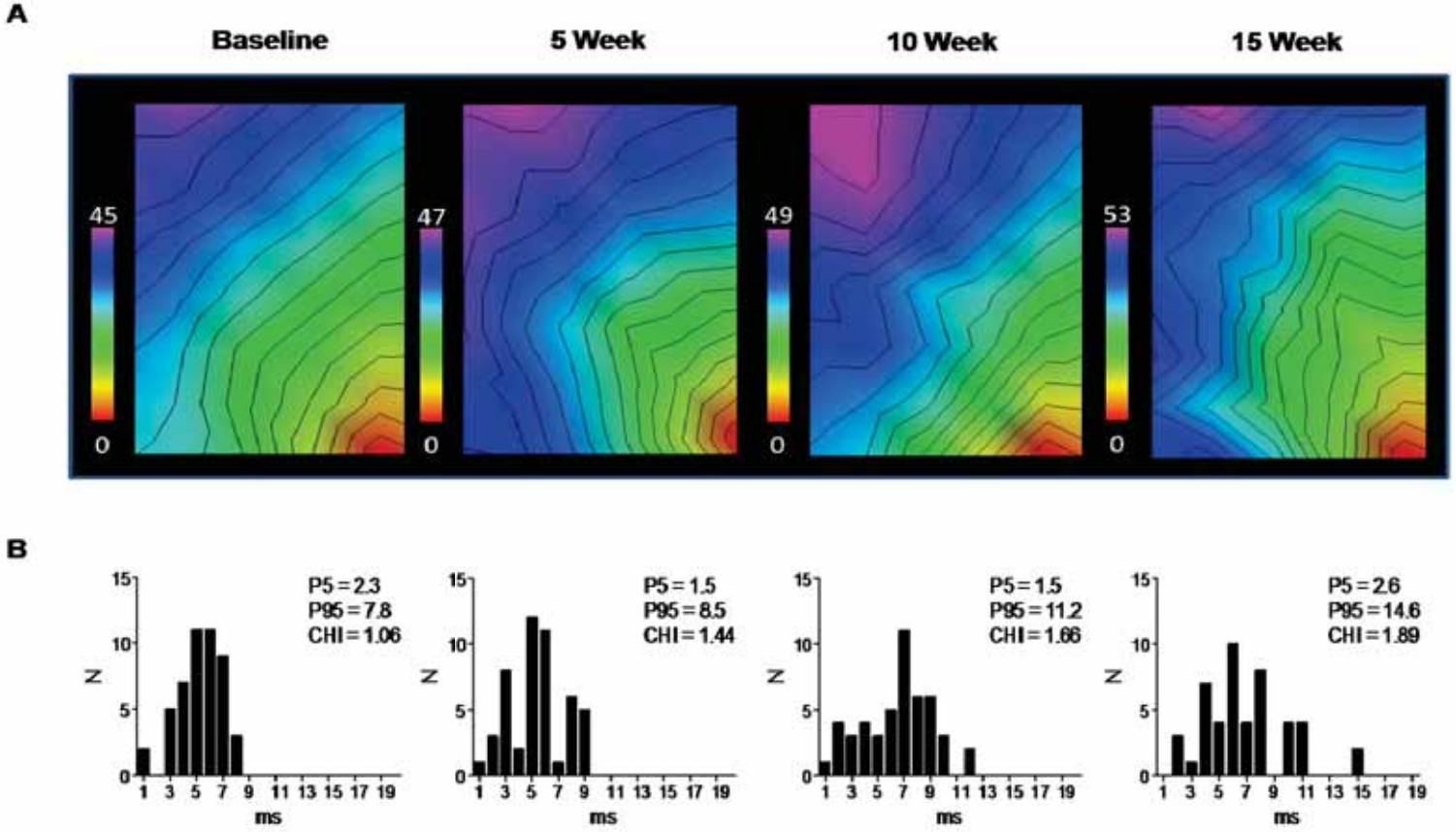
20 sheep were used to study structural and functional remodeling at baseline, 5, 10 and 15 weeks of hypertension. The remaining 12 sheep were utilized for closed chest conscious electrophysiological evaluations over 15 weeks with and without hypertension.

Figure 2: “One Kidney-One Clip” Blood Pressure Profile



Blood pressure remained stable from baseline to post nephrectomy. Both systolic and diastolic rose sharply after renal clamping to reach a plateau around 4 weeks duration. Error bars denote standard deviation.

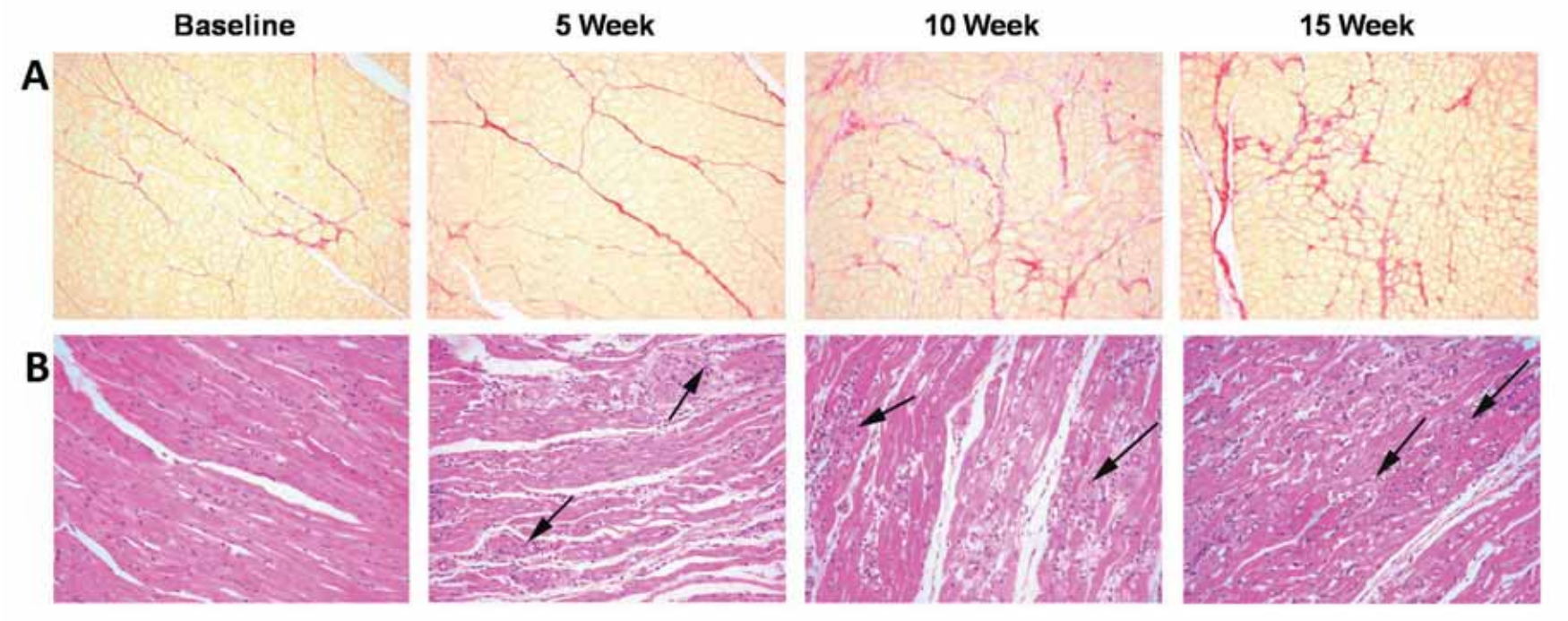
Figure 3: Representative (A) Activation Maps and (B) Corresponding Phase Histograms at Different



P5-95, absolute phase delay; CHI, conduction heterogeneity index; Phase represented in ms per 5mm

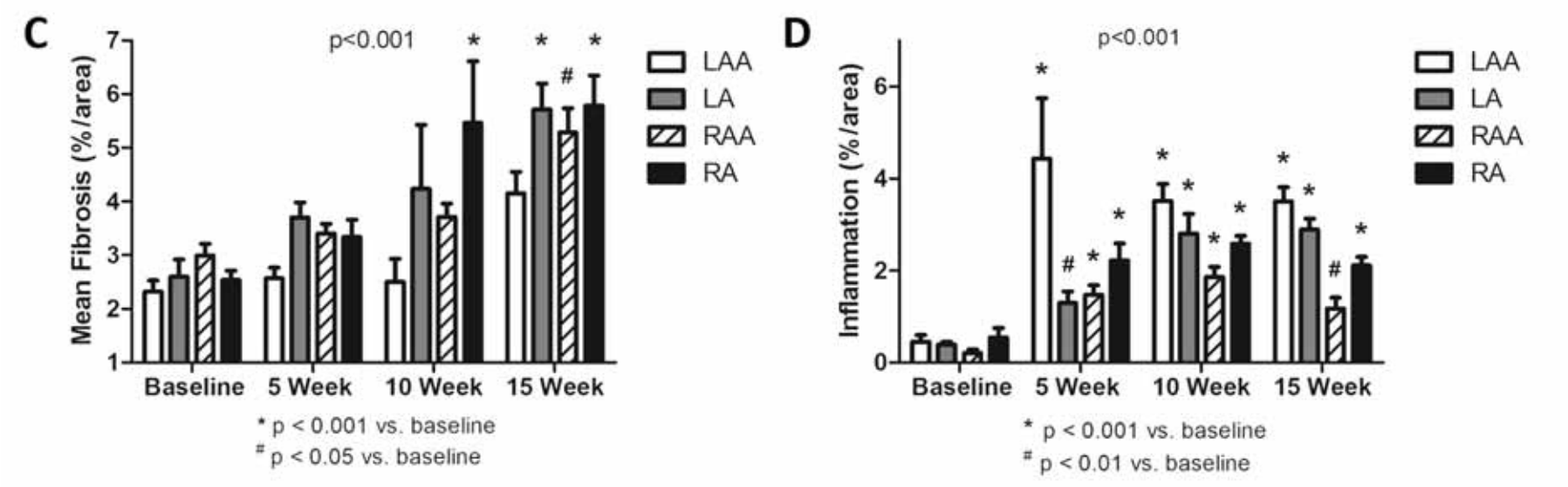
(A) These are examples of activation maps when paced at the left atrial appendage at 400ms. Isochrones of 3 ms have been superimposed. Increasing isochronal crowding is observed with longer plaque activation time from baseline through to 15 weeks of hypertension. (B) These phase histograms from corresponding stages of hypertension illustrate the increasing absolute inhomogeneity of conduction (P_5-P_{95}) and conduction heterogeneity index (CHI) from baseline through to 15 weeks of hypertension.

Figure 4A & B: Atrial Structural Remodeling in Hypertension



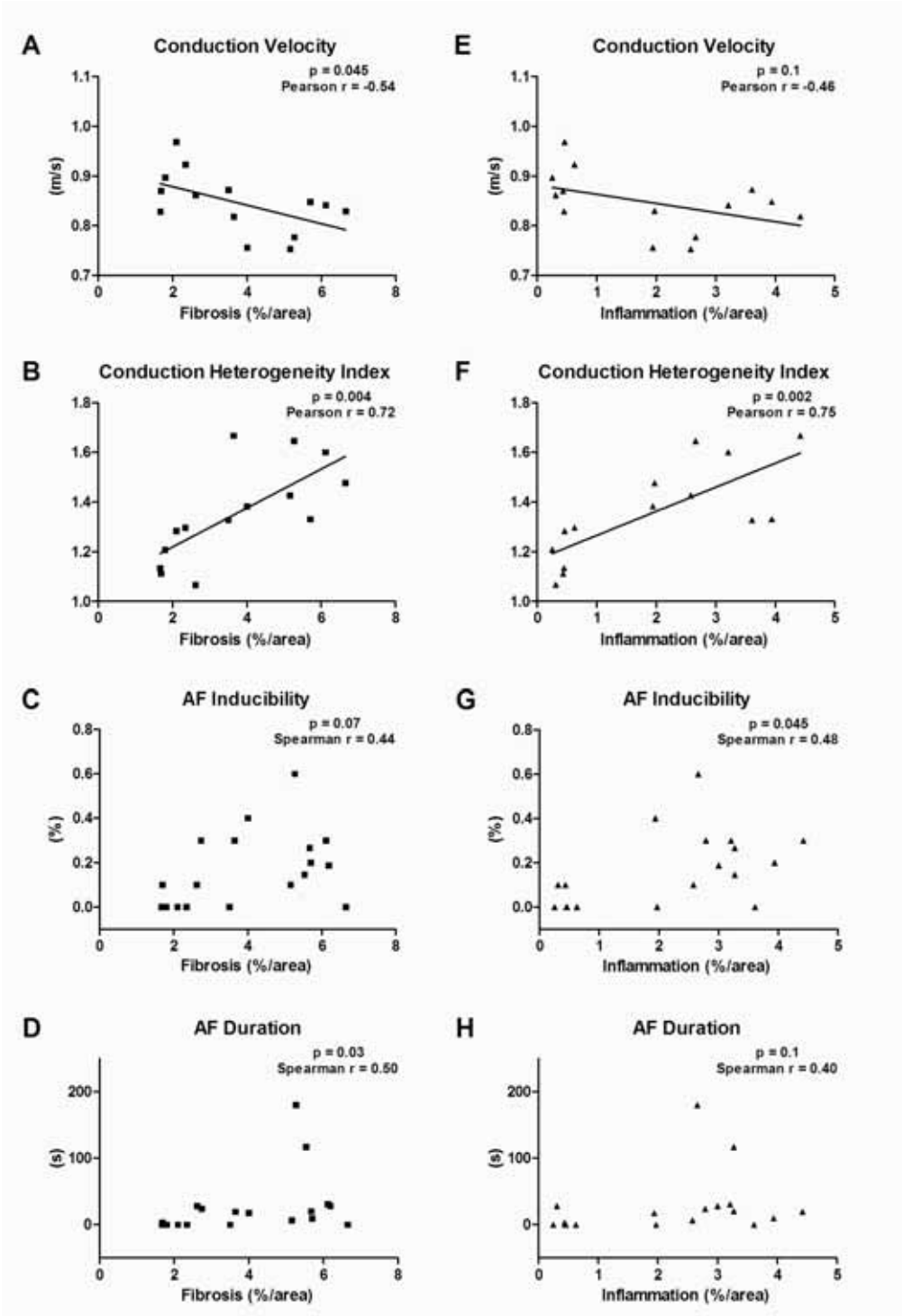
Representative picrosirius red stains from the LA are shown in panel A illustrating increasing interstitial collagen (red staining) from little to extensive deposition from baseline to 15 weeks of hypertension [Magnification x250].
Representative H&E stains from the LA are shown in panel B illustrating the early increase in inflammatory infiltration from 5 weeks of hypertension [Magnification x250].

Figure 4C & D: Atrial Structural Remodeling in Hypertension



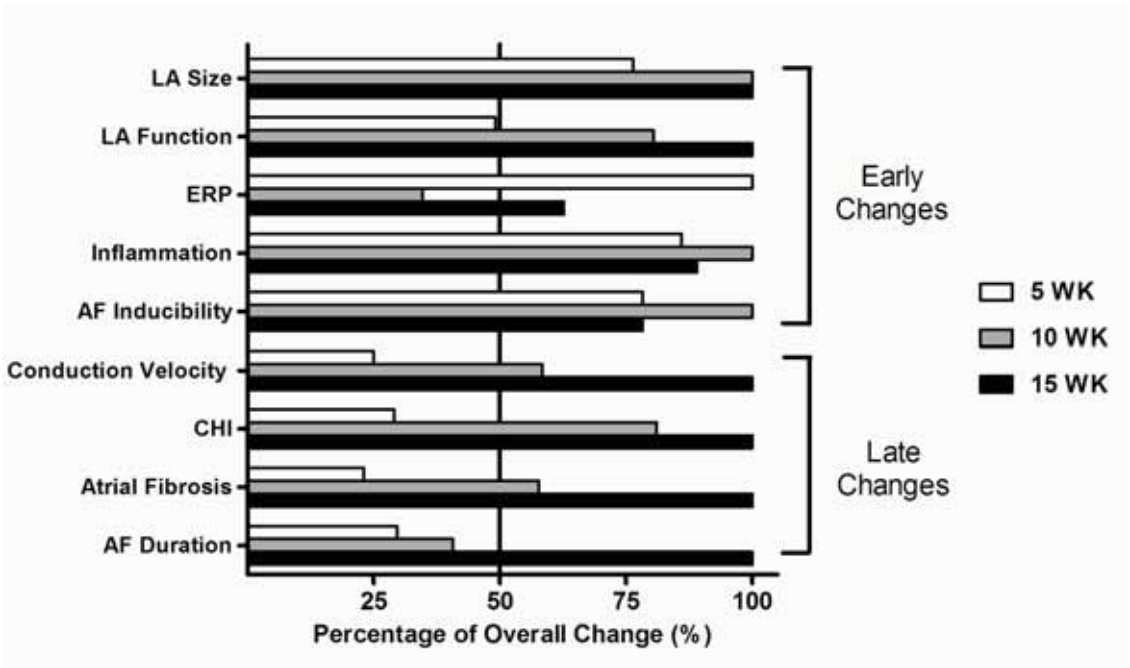
Panel C shows the increasing percentage area of atrial interstitial fibrosis (mean±SEM) over 15 weeks of hypertension ($p<0.001$). Panel D illustrates the early increase in inflammatory infiltrates (mean±SEM) during progressive hypertension ($p<0.001$). P for site specific comparisons at different time points to respective baseline values are also included for both panel C and D.

Figure 5: Atrial Electro-structural Correlate



Significant correlation was seen between atrial fibrosis and conduction velocity/heterogeneity and AF duration (Panels A-D). Corresponding analysis of atrial inflammation showed significant correlation with conduction heterogeneity index and AF inducibility only (Panels E-H).

Figure 6: Remodeling Occurs at Different Time Domains



LA; left atrial, ERP; effective refractory period, CHI; conduction heterogeneity index.

This graph illustrates the percentage of total change from baseline in the various parameters at different hypertensive time-points. Changes in LA size, LA function, ERP and inflammation occurred early (~50% or more seen by 5 weeks) contributing to increased AF inducibility. Changes in atrial fibrosis with its consequent conduction abnormalities occurred later (10 weeks) leading to longer AF episodes.

Chapter Five

Atrial Remodeling in an Ovine Model of Anthracycline-induced Non-ischemic Cardiomyopathy: "Remodeling of the Same Sort"

5.1 INTRODUCTION

Both heart failure (HF) and atrial fibrillation (AF) have emerged as epidemics in this new millennium. The complex causal association and compounding interaction between these two conditions often lead to more detrimental consequences as compared to each condition in isolation.[353-355] At present, our understanding of the patho-physiological mechanisms of this link remain limited. In particular, all pre-clinical studies on atrial remodeling in HF have been confined to a single animal model using rapid ventricular pacing.[200, 297, 359, 438] No single model can be representative of the complex clinical syndrome of HF in humans where the different underlying causes have been shown to portend different prognostic value.[360] Furthermore, tachycardia in itself has been well established to result in remodeling of its own[102] and cessation of rapid pacing is associated with the rapid reversibility of atrial and ventricular function, atrial electrical and ionic remodeling as well as ventricular structural changes; all features that are not reflective of the natural history of most types of HF.[438, 444, 445]

In order to determine whether the changes observed in prior studies were specific to the model used or a result of HF, we undertook detailed evaluation of the atrial electrical, functional and structural abnormalities that develop in a recently characterized ovine model of doxorubicin-induced non-ischemic cardiomyopathy.[446, 447]

5.2 METHODS

Fourteen sheep formed the study protocol; following initial acclimatization, animals were allocated sequentially to either control (n=7) or heart failure (n=7) group. All procedures were conducted in accordance with the guidelines outlined in the "Position of the American Heart Association on Research Animal Use" adopted on November 11, 1984 by the American Heart Association. Approval for the performance of the study was provided by the Animal Ethics Committees of the Institute of Medical and Veterinary Services and the University of Adelaide, Adelaide, Australia.

5.2.1 Doxorubicin Non-ischemic Cardiomyopathy Model

The establishment and characterization of this model has been described elsewhere.[447] In brief, all animals in the HF group underwent creation of a small 3cm pericardial window to avoid inflammatory pericardial effusion (noted in previous studies) at baseline prior to cardiac imaging and doxorubicin dosing.[446]

Doxorubicin (1 mg/kg) was infused over 30 minutes via catheterization of the left-sided coronary arteries (Amplatz AL1 catheter, Cordis Corp, Miami, FL, USA) under fluoroscopic guidance at fortnightly intervals. A total of 3 to 4 doses were required to achieve at least moderate left ventricular systolic dysfunction without evidence of myocardial infarction with histological validation.[447]

5.2.2 General Anesthesia

General anesthesia was utilized for all investigational (trans-thoracic echocardiography - TTE and cardiac magnetic resonance imaging - CMR) and interventional procedures (pericardial window, cardiac catheterization and electrophysiological studies). Sodium thiopental (15-20mg/kg) was used for induction to facilitate endotracheal intubation and isoflurane (2-4%) in 100% oxygen was used for maintenance. Invasive blood pressure, heart rate, end-tidal CO_2 and temperature were continuously monitored. Specifically, mechanical ventilation was maintained during CMR scans to facilitate ECG-gated image acquisition with adequate breath-holding. During electrophysiological and hemodynamic assessments, the dose of isoflurane was fixed at 2% with end-tidal CO2 kept at 40 mmHg through mechanical ventilation.

5.2.3 Cardiac Functional Assessments

CMR was utilized to assess both left atrial and ventricular volumes and ejection fraction at baseline and prior to the euthanasia studies (Siemens Sonata 1.5 Tesla & Leonardo workstation, Siemens Medical Solutions, Erlangen, Germany). Slice thickness through the atria and ventricles were 6mm and 10mm respectively without any inter-slice gap. TTE was used to monitor cardiac function serially during the doxorubicin dosing protocol (Acuson XP-128, 4 MHz probe, Siemens Medical Solutions, Erlangen, Germany).

5.2.4 Electrophysiological Study

Open chest electrophysiological studies were performed via bilateral thoracotomy approach whereby physiological arterial blood gases and body temperature were maintained throughout. A custom designed 128-electrode epicardial plaque with 5mm inter-electrode distance was then applied to the RA, LA and traversing the Bachmann's bundle before being attached to a computerized recording system (LabSystem Pro, Bard Electrophysiology, Lowell, MA, USA), as previously described.[448] Surface-ECG and overlapping bipolar electrograms were continuously monitored and stored for off-line analysis. Electrograms were filtered from 30-500Hz, and measured with computer-assisted calipers at a sweep speed of 200mm/s.

5.2.4.1 Atrial ERP

Atrial ERP was measured at twice diastolic threshold at cycle lengths (S1) of 500, 400, 300 and 200ms from six sites (right atrial appendage: RAA, right atrial free wall: RAFW: right atrial Bachmann's Bundle: RABB, LAA, LAFW and LABB). Eight basic (S1) stimuli were followed by a premature (S2) stimulus in 10ms decrement. Atrial ERP was defined as the longest S1-S2 interval not resulting in a propagated response. Each measurement was repeated twice and if the variability was greater than 10ms, three further measurements were taken and the total averaged. ERP rate adaptation was calculated as the difference between ERP_{500} and ERP_{200}. The coefficient of ERP variation (SD/mean x 100%) was determined in each atrium to assess ERP heterogeneity.

5.2.4.2 Atrial Conduction

Conduction was assessed during stable S1 pacing at cycle lengths of 500, 400, 300 and 200ms from four pre-specified sites at the RAA, RAFW, LAA and LAFW. Activation maps were created using semi-automated custom made software (Nucleus Medical, Adelaide, Australia). Each annotation was manually verified with the local activation time annotated to the peak of the largest amplitude deflection on bipolar electrograms. Local conduction velocity was calculated from the local vectors within each triangle of electrodes as previously

described.[426, 449] A mean conduction velocity can then be derived for each activation map.

Heterogeneity of Conduction

Conduction heterogeneity was assessed using established phase mapping technique during S1 pacing.[427] In brief, the largest activation time difference between every four adjacent electrodes was first determined and divided by inter-electrode distances. The largest value at each site was then used to create a phase map, with values also displayed as a histogram. Absolute conduction phase delay was calculated by subtracting the 5th from the 95th percentile of the phase-difference distribution (P_{5-95}). The conduction heterogeneity index was then calculated by dividing the absolute phase delay by the median (P_{50}).

5.2.4.3 Direction-dependent Conduction Abnormalities

Pacing from the atrial appendages (RAA, LAA) produced a cranial to caudal wavefront propagation whilst pacing from the atrial free walls (RAFW, LAFW) resulted in a caudal to cranial wavefront propagation. To assess for direction-dependent conduction abnormalities, we compared both conduction velocity and conduction heterogeneity index during pacing between the respective appendages and free walls in both groups of animals.

5.2.4.4 P-wave Duration

P-wave duration was averaged over ten consecutive beats as a surrogate marker of inter-atrial conduction time and measured on lead II of the surface-ECG.

5.2.4.5 AF Inducibility and Duration

AF induction was assessed with rapid atrial pacing at the RAA and during ERP testing. Rapid pacing started at 200ms cycle length and was decreased in 5ms intervals until either AF was initiated or there was loss of 1:1 atrial capture. A fixed rate of decrement was used at 5ms per 3s to ensure consistency. This protocol of AF induction was repeated ten times. During ERP testing, induced AF episodes were carefully documented with percentage of inducibility taken as the number of AF episodes over the total number of S1-S2 drivetrains delivered. The overall inducibility was the average of inducibility during both ERP testing and rapid decremental pacing.AF was defined as a rapid irregular atrial rhythm of ≥2s. Mean duration of AF episodes were derived from the average of all induced AF episodes during ERP testing and following decremental pacing.

5.2.5 Structural Analysis

At the end of the electrophysiological studies, animals were euthanized using pentobarbital and the hearts promptly removed for structural analysis. Both

atria and their appendages were separated and immersed fixed with 10% formalin and paraffin embedded for subsequent evaluation. Sections were stained with picrosirius red to demonstrate the extracellular matrix in a masked protocol. Five random stained sections from each individual atrium and appendage of every animal were digitally captured (20 sections/animal) with an area of red selected for its color range and the proportional area of tissue with this range of color quantified. Calculation of the proportional area was then determined using image analysis (Analytical imaging Station, Version 6.0, Imaging Research Inc., St. Catherines, Ontario, Canada).

5.2.6 Statistical Analysis

All continuous variables are reported as mean±standard deviation and assessed for normality utilizing the Shapiro-Wilk test. To improve presentation clarity, mean±SEM was utilized in figures 1 and 2. Data that were normally distributed were compared using the Student's t-test. Data that were not normally distributed were compared using the Wilcoxon rank-sum tests. Analyses of differential effects of HF and over various atrial locations were performed using ANOVA. Repeated measures ANOVA was used when data was analyzed across >2 cycle lengths or >2 time-points. Post-hoc Tukey's test was used to compare baseline vs. final values for fractional shortening and complete blood count analyses. Statistical significance was established at $p<0.05$.

5.3 RESULTS

Due to the loss of one HF animal during induction of general anesthesia for final cardiac magnetic resonance scan, we only included data from the remaining 13 animals (6 HF and 7 controls). Both HF and control groups were well matched for size with no differences seen in their weight at baseline (52±6 vs. 48±7 kg; p=0.3) or at euthanasia (54±7 vs. 49±5kg; p=0.1). Detailed animal characteristics are presented in Table 1. The HF group received an average doxorubicin dose of 3.8±0.5mg/kg resulting in moderate global reduction in left ventricular function on both TTE and CMR assessments. Cardiac index was significantly lower in the HF group but no differences were seen in heart rate, pulmonary capillary wedge pressures and heart weight. Specifically, the development of left ventricular dysfunction was progressive with gradual decline of fractional shortening from baseline to before final doxorubicin dosing to 6 weeks after the last doxorubicin dose at the time of euthanasia (33.0±2.1 vs. 26.2±2.7 vs. 20.9±1.7%, $p<0.001$ for trend and $p<0.05$ for baseline vs. final). No change in fractional shortening were seen in the controls from baseline to the time of euthanasia (31.9±2.8 vs. 32.5±3.3; p=0.8).

All HF animals suffered minor systemic effects of doxorubicin including temporary wool loss, diarrhea and temporary decrease in hemoglobin, total white cell and platelet counts. Specifically, diarrhea occurred in <25% of animals and lasted 1-2 days at most; usually occurring 2-3 days after the 3rd or 4th dose

of doxorubicin. This did not require any treatment as no electrolyte abnormalities were detected. The complete blood count profile from baseline to just prior to second, third, fourth doxorubicin dosing and pre-euthanasia final examinations were as follows: Hemogloblin (99±6 vs. 97±7 vs. 93±9 vs. 91±8 vs. 98±8; p=0.03 for trend and p=NS for baseline vs. final); total white cell count (6.2±1.3 vs. 5.1±2.0 vs. 4.1±0.9 vs. 3.4±1.3 vs. 4.5±1.4; p<0.001 for trend and p<0.05 for baseline vs. final); platelet count (250±86 vs. 172±59 vs. 183±53 vs. 201±68 vs. 184±51; p=0.002 for trend and p=NS for baseline vs. final). Only 2 out of the 6 HF animals demonstrated respiratory difficulty in the last 1-2 weeks prior to euthanasia study. This was managed with small doses of frusemide (subcutaneous, 0.5-1 mg/kg) and fluid restriction.

5.3.1 Atrial Functional Remodeling due to Doxorubicin Cardiomyopathy

There was significant atrial dilatation and reduced LA ejection fraction in animals with HF compared to controls (Table 1).

5.3.2 Atrial Electrical Remodeling due to Doxorubicin Cardiomyopathy

5.3.2.1 Atrial Refractoriness

The HF group demonstrates higher ERP in both atria as compared to the controls (RA: 191±37 vs. 168±30ms; p=0.04, LA: 154±37 vs. 133±24ms; p=0.03;

respectively, S1=300ms) at all cycle lengths (Figure 1A) and locations (Figure 1B) with preserved physiological rate adaptation (RA: 21±19 vs. 23±14ms, p=0.5; LA: 15±17 vs. 20±21ms, p=0.4). Heterogeneity of ERP did not differ in the HF versus control group at all cycle lengths (RA: 19±3 vs. 16±7%, p=0.5; LA: 24±4 vs. 18±8%, p=0.2; respectively, S1=300ms).

5.3.2.2 Atrial Conduction

Conduction velocity is significantly slower in both atria of the HF group as compared to the control animals at all pacing cycle lengths (RA: 0.72±0.01 vs. 0.87±0.06m/s, LA: 0.75±0.02 vs. 0.92±0.03m/s respectively; both $p<0.001$, Figure 2A). Phase mapping shows an increased absolute range of conduction delay (P_{5-95}: 2.3±0.3 vs. 1.7±0.1ms/mm; $p<0.001$) and higher conduction heterogeneity index (P_{5-95}/P_{50}: 1.41±0.05 vs. 1.21±0.02; $p<0.001$) in the HF group (Figure 2B). Representative activation maps during S1 pacing at 300ms from the LA free wall are shown in Figure 3A illustrating slower conduction in the HF example with longer total activation time of 49 versus 42ms. Figure 3B shows their corresponding phase histogram whereby increased conduction heterogeneity index is evident in the HF example.

5.3.2.3 Direction-dependent Conduction Abnormalities

No significant differences in direction-dependent conduction velocity (RAA vs. RAFW: 0.88±0.11 vs. 0.85±0.18 m/s, p=0.5; LAA vs. LAFW: 0.92±0.12 vs. 0.92±0.13 m/s, p=0.6) and conduction heterogeneity index (RAA vs. RAFW: 1.22±0.33 vs. 1.22±0.22, p=0.9; LAA vs. LAFW: 1.16±0.33 vs. 1.22±0.23, p=0.7) were seen in the control group. In the HF animals, direction-dependent conduction slowing was found in the caudal to cranial direction although this was statistically significant in the RA only (RAA vs. RAFW: 0.74±0.06 vs. 0.69±0.05 m/s, p=0.002; LAA vs. LAFW: 0.74±0.11 vs. 0.73±0.08 m/s, p=0.3). However, conduction heterogeneity index was significantly higher in the caudal to cranial direction in both atria of the HF group (RAA vs. RAFW: 1.29±0.22 vs. 1.43±0.15, p=0.02; LAA vs. LAFW: 1.39±0.24 vs. 1.61±0.44, p=0.03).

5.3.2.4 P wave Duration

HF results in longer P wave duration as compared to control animals (65±5 vs. 56±6ms; $p<0.05$)

5.3.2.5 AF Inducibility and Duration

The HF group demonstrated a trend towards a greater AF inducibility compared to the controls (27±31 vs. 3±4%; p=0.08). Specifically, with the rapid

decremental pacing protocol, AF was inducible in 5 out of 6 HF animals as compared to only 2 out of 7 control animals. In addition, HF animals were able to sustain significantly longer induced AF episodes (16±22 vs. 2±3s; p=0.04).

5.3.3 Atrial Structural Remodeling due to Doxorubicin HF

There is increased interstitial collagen deposition in the HF atria and their respective appendages as compared to the controls. This is evident from the picrosirius red stains (Figure 4) as well as quantitatively, with significantly higher percentage area of interstitial fibrosis (Figure 5).

5.4 DISCUSSION

In this model of persistent non-ischemic cardiomyopathy with demonstrable moderate global systolic dysfunction we observed significant atrial remodeling characterized by:

1. Left atrial enlargement with impaired LA mechanical function;
2. Structural abnormalities characterized by increased interstitial fibrosis;
3. Significant conduction slowing with increased heterogeneity associated with prolonged P wave duration;
4. Increased refractoriness with preserved heterogeneity;

5. Perhaps as a result of these abnormalities these animals with persistent HF had longer AF episodes.

The remarkable similarity of these findings to previous studies of rapid ventricular pacing HF suggests a common end result dominated by structural changes and resultant conduction abnormalities in the substrate for AF in HF.

5.4.1 Rapid Ventricular Pacing Model of HF

Since the initial description of experimental HF by chronic rapid pacing in 1962, the model has been utilized widely in HF studies.[358] To date, all studies on HF related atrial remodeling have been based on this single animal model of rapid ventricular pacing leading to the following concerns. First, whether rapid ventricular pacing leads to rate related remodeling of the atria or that it increases the risk of AF due to the resultant ventricular dyssynchrony is not known.[450] Second, the rapid reversibility of atrial and ventricular function, atrial electrical and ionic remodeling as well as ventricular structural changes on cessation of rapid ventricular pacing is not physiologically comparable to most types of cardiomyopathy.[438, 444, 445] Of note, the underlying mechanisms responsible for HF remain elusive. Finally, the rapid pacing model is not representative of all types of cardiomyopathy in the HF syndrome, mandating evaluation of different HF models. In addition to these potential disease related limitations of the rapid ventricular pacing model of HF, technical limitations

prohibit its use for further study of HF physiology due to: [1] the requirement of ongoing rapid ventricular pacing to sustain HF which precludes interventional studies on reverse remodeling in both the atrial and ventricular levels, limiting studies to examine "pre-treatment" effects instead;[451, 452] and [2] the presence of the pacing system which prevents the use of state of the art CMR imaging modality, a gold standard measure of cardiac function capable of additional structural evaluation using novel applications.

5.4.2 Advantages of Doxorubicin Non-Ischemic Cardiomyopathy Model

The clinical entity of non-ischemic cardiomyopathy encompasses a wide range of etiologies which includes exposure to cardio-toxic agent such as doxorubicin. This relationship is dose dependent with incidence of cardiomyopathy estimated at up to 48%.[453, 454] Long term follow-up studies in humans have demonstrated the progressive and irreversible nature of left ventricular dysfunction in anthracycline-induced cardiomyopathy which is typically refractory to conventional therapy.[454-456] Animal model of cardiomyopathy secondary to systemic administered doxorubicin characterized by severe cardiac dilatation with fluid retention and activation of the neurohormonal systems has been utilized in HF research since the 1980s.[457]

Systemic administration of doxorubicin has been associated with substantial side effects and mortality. However, recent experience using intra-coronary delivery of doxorubicin has resulted in more favorable tolerability, survival and reproducibility with CMR and histo-pathological changes representative of non-ischemic cardiomyopathy seen in humans.[446, 447] While the toxic effects of doxorubicin on the atria is not known and could not be quantified, the usage of doxorubicin HF model in studies evaluating atrial remodeling has additional advantages including: more permanent non-reversible HF, better suitability for interventional studies examining reverse remodeling and compatibility to CMR imaging modality.

5.4.3 Remodeling of the Same Sort?

This study presents important detailed information on atrial remodeling in a recently characterized ovine model which complements existing literature. In the canine rapid ventricular pacing model of HF, Li and co-workers demonstrated the promotion of sustained AF by increased atrial interstitial fibrosis and conduction heterogeneity with no changes in atrial refractoriness and conduction velocity after 5 weeks of HF.[200] In addition, evaluation of remodeling using electroanatomic mapping in humans with both ischemic and non-ischemic cardiomyopathy demonstrated similar evidence of structural changes, conduction abnormalities and increased refractoriness.[302] In contrast, more recent work in canine chronic rapid ventricular pacing HF model of 4

months duration showed ion current remodeling distinct from short term studies together with reduced atrial ERP and increased interstitial fibrosis.[458] This was in keeping with the work by Workman and colleagues whereby atrial cellular ERP was found to be abbreviated in humans with left ventricular dysfunction.[459] The variability in ERP changes could be explained by differences in species, models, method of electrophysiological studies (whole organ vs. cellular) and the chronicity or severity of HF. Nevertheless, our findings of structural changes of atrial fibrosis and the consequent conduction abnormalities leading to its arrhythmogenesis in the doxorubicin HF model appears similar to other animal models and human cardiomyopathies; implicating a likely common final substrate.

In addition, very similar changes have been observed in patients with other conditions known to be established substrates predisposing to AF including sinus node disease, atrial septal defects and mitral stenosis.[302-304, 377] Indeed, the pivotal contributory role of increased atrial interstitial fibrosis with its resultant conduction abnormalities has also been demonstrated in other animal models such as hypertension and mitral regurgitation.[236, 448] Recently, Stiles at al. have also demonstrated similar changes in patients with "lone AF" implicating their role in the substrate for AF.[169] Taken together with all these studies, this work implicates structural remodeling with increased atrial fibrosis and its electrophysiological consequences as universal in different arrhythmogenic

substrates. Due to incomplete understanding of the underlying patho-physiological mechanisms in different AF substrates, atrial fibrosis appears to represent “remodeling of the same sort”.

5.4.4 Clinical Significance

By affirming the importance of interstitial fibrosis in atrial arrhythmogenesis in different HF models, this study provides further impetus to develop novel selective therapies specifically targeting the atrial structural changes in HF since agents shown to attenuate HF-induced atrial structural remodeling, such as inhibitors of the renin-angiotensin-aldosterone system and 3-hydoxy-3-methyglutaryl coenzyme A (HMG-CoA) reductase inhibitors, have been proven to be beneficial in reducing AF in HF.[429, 451, 452, 460] In addition, novel therapies whether anti-fibrotic agents or cell/gene based may also be useful in other AF substrates which demonstrate similar structural remodeling.

5.4.5 Study Limitations

Systemic or local effects of direct doxorubicin toxicity on the atria could not be teased out. This model may not be representative of all types of human HF but it presents an alternate HF model which can be used to further improve the mechanistic understanding of the heterogeneous clinical HF syndrome. The control animals were not sham operated or treated. The development of clinical

AF is recognized to occur through a complex interaction of triggers, perpetuators and substrate. This study focused on the atrial substrate and did not evaluate other contributing factors to the development of AF. Despite the observed structural and electrical changes, induced AF episodes in HF animals were not particularly sustained. Additional cellular measurements of action potential duration and ion currents were not performed.

5.5 CONCLUSION

This model of non-ischemic cardiomyopathy due to doxorubicin is characterized by significant atrial remodeling with increased fibrosis and its resultant slowed/heterogeneous conduction, atrial dysfunction and increased refractoriness associated with more sustained AF. These findings appear the "same sort" to previous models of HF and in other predisposing conditions thereby implicating a final common substrate leading to the development of AF.

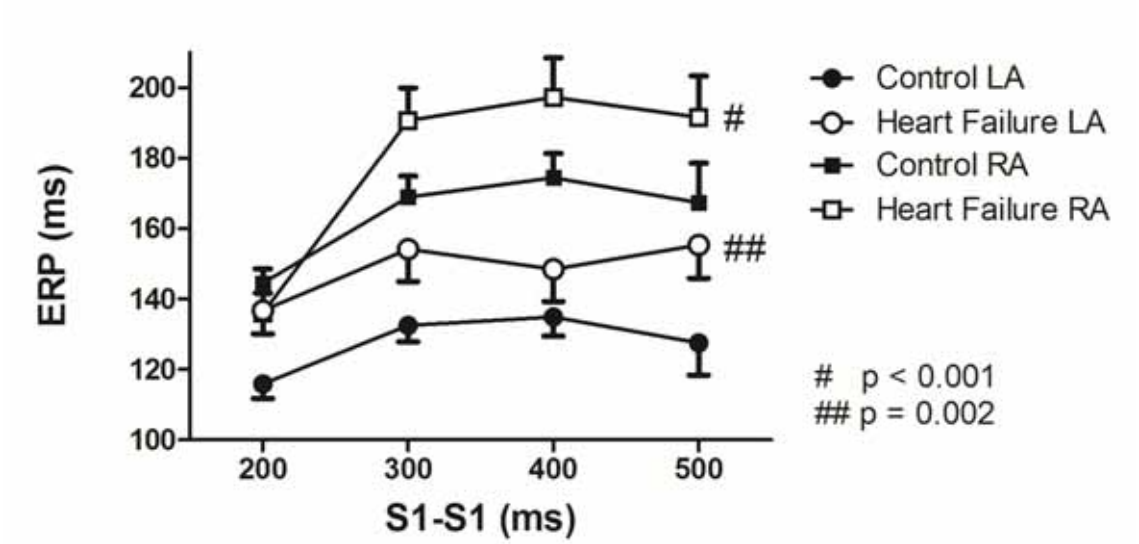
Table 1: Animal Characteristics

	Heart Failure (n=6)	Controls (n=7)	P
Heart Rate (beats/min)	105±8	104±18	0.9
PCWP (mmHg)	11.2±5.2	9.8±1.5	0.6
Cardiac Index	2.1±0.2	2.4±0.1	0.03
Heart/Body Weight (g/kg)	5.6±0.9	6.3±0.4	0.2
Echocardiography			
LV Fractional Shortening (%)	20.9±1.7	32.5±3.3	0.003
CMR			
LV End Diastolic Volume (ml)	76.2±15.6	70.8±7.0	0.5
LV End Systolic Volume (ml)	46.5±9.5	38.3±6.7	0.2
LV Ejection Fraction (%)	37.1±4.6	46.4±4.1	0.003
LA End Diastolic Volume (ml)	39.8±2.0	28.7±6.3	0.02
LA End Systolic Volume (ml)	31.1±2.3	19.2±2.4	<0.001
LA Ejection Fraction (%)	23.4±4.4	32.1±2.8	0.005

LA, left atrial; LV, left ventricular; PCWP, pulmonary capillary wedge pressure.

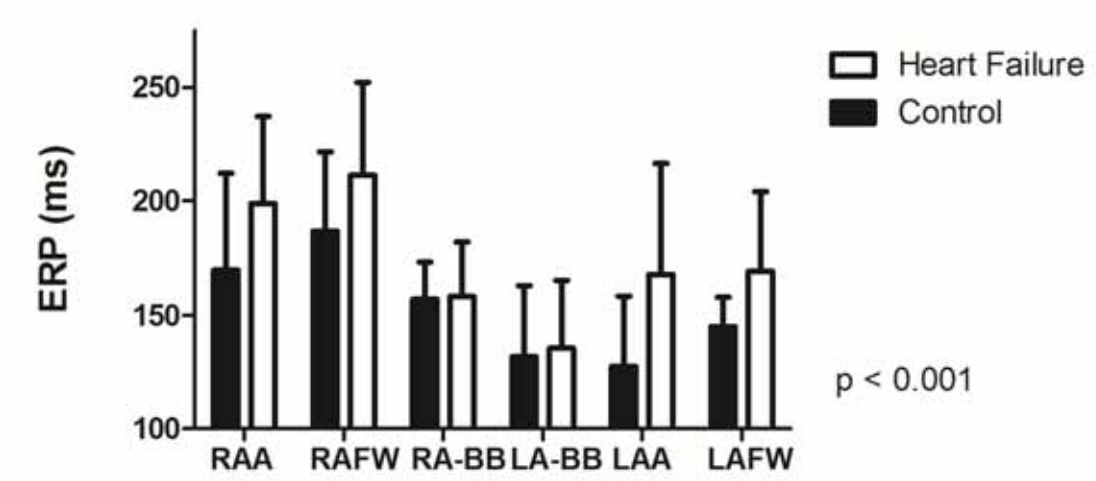
Figure 1: Atrial ERP

A Mean ERP at different pacing cycle lengths



ERP (mean±SEM) is uniformly higher in the HF animals at all pacing cycle lengths compared to controls. [LA; left atrial, RA; right atrial]

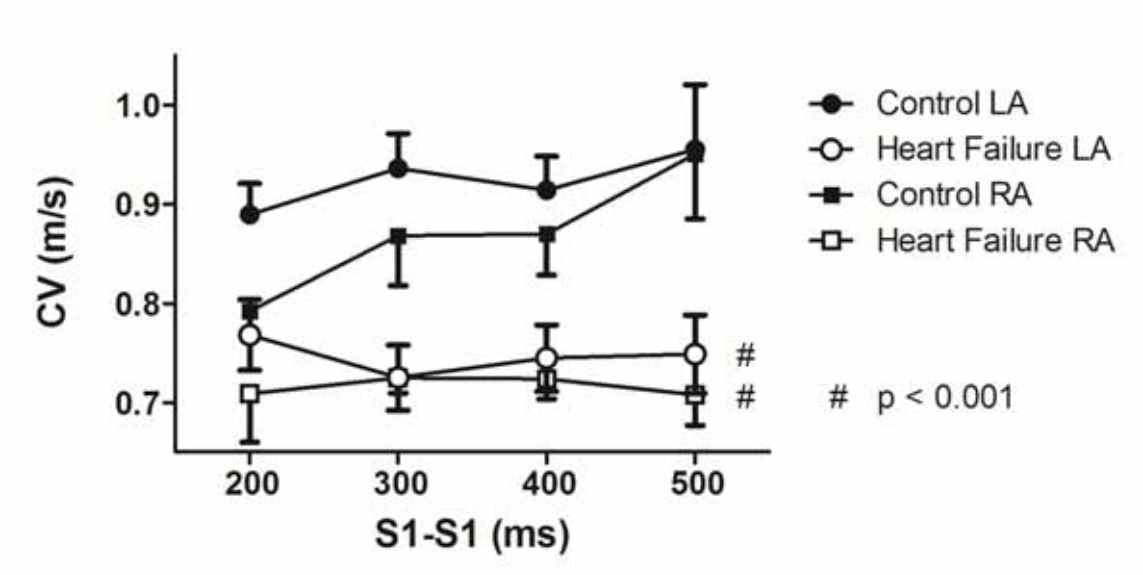
B Mean ERP by pacing locations (S1 = 300ms)



Similarly, ERP (mean±SEM) is higher in the HF group when analyzed by pacing sites. This graph illustrates the difference between the two groups during pacing cycle length of 300ms.

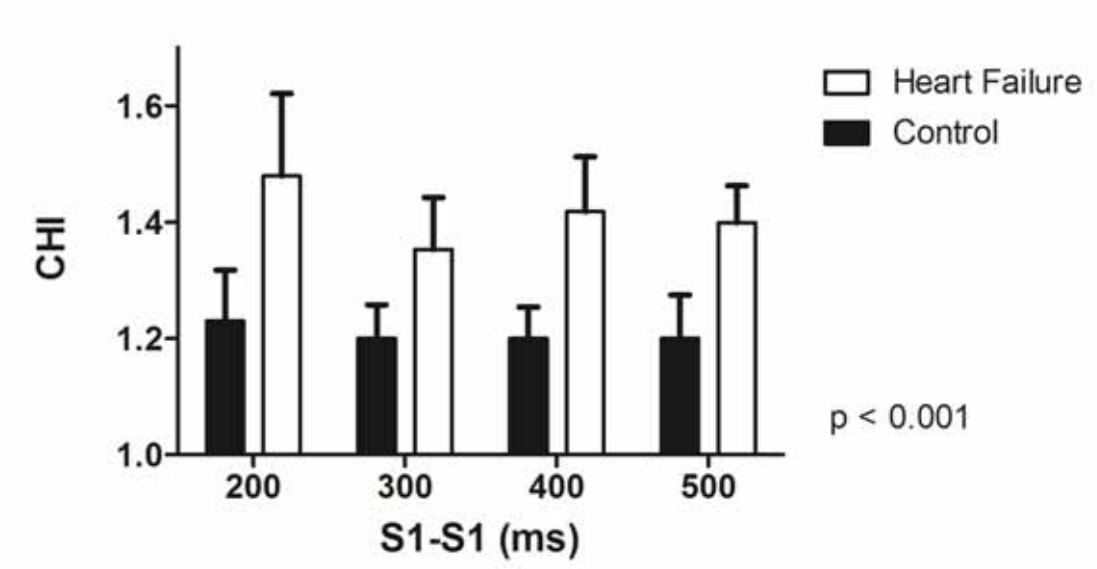
Figure 2: Atrial Conduction

A Conduction Velocity



HF results in slower conduction velocity (mean±SEM) at all pacing cycle lengths. [LA; left atrial, RA; right atrial]

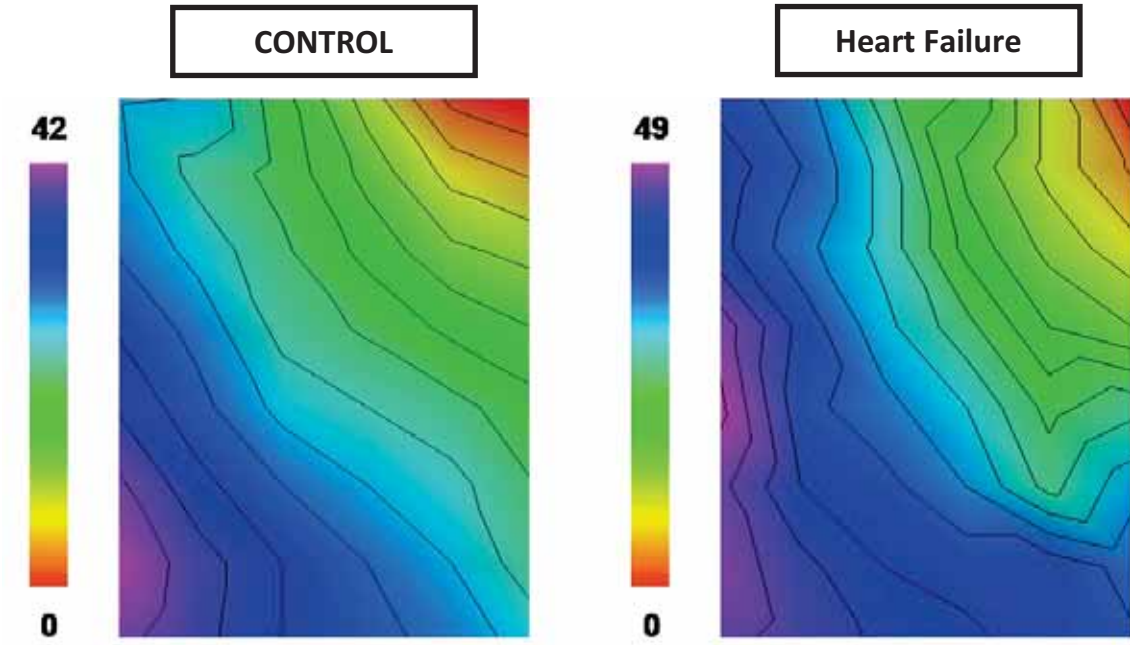
B Conduction Heterogeneity Index



Phase mapping analysis shows higher conduction heterogeneity (mean±SEM) in the HF animals at all drivetrains.

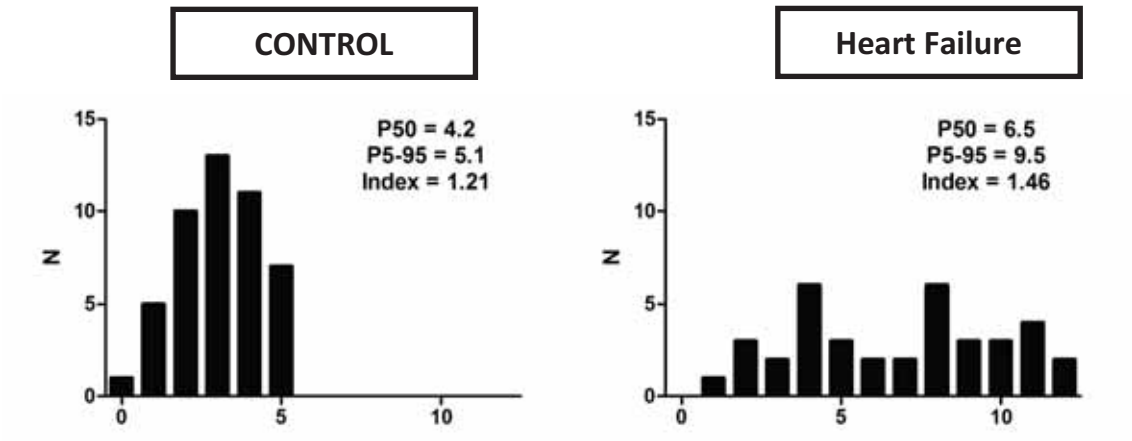
Figure 3: Atrial Activation Maps and Phase Histograms

A Representative Activation Maps



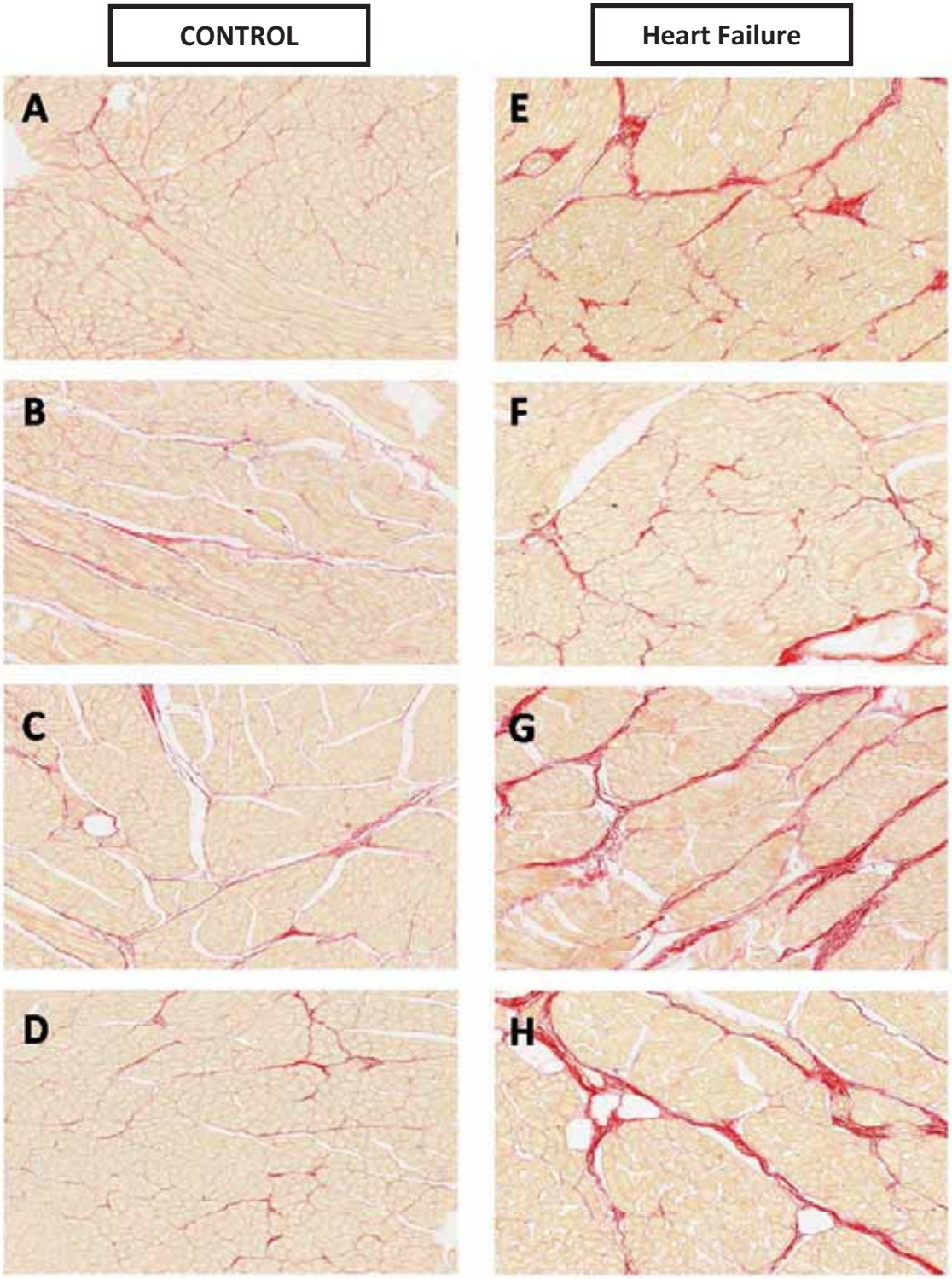
Representative activation maps from control and HF atria during S1 pacing from the LA free wall at 300ms. Isochrones have been constructed at 3ms intervals. Isochronal crowding is evident in the HF atrium indicative of slower conduction.

B Corresponding Phase Histograms



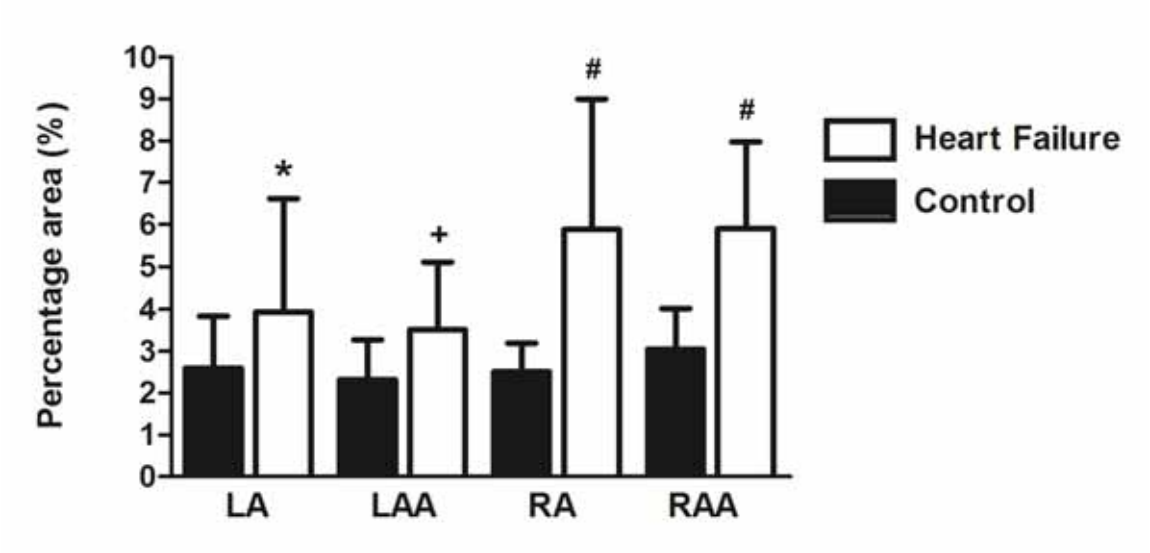
Corresponding phase histogram shows increased conduction heterogeneity in the HF example.

Figure 4: Representative Picrosirius Red Sections



In control LA; left atrial(A), LAA; left atrial appendage(B), RA; right atrial(C) and RAA; right atrial appendage(D), little collagen (red staining) is present within the interstitium, while extensive interstitial fibrosis is noted in HF LA(E), LAA(F), RA(G) and RAA(H). [Magnification x350]

Figure 5: Quantification of Collagen Matrix



* P=0.02, $^{+}$P=0.001, $^{\#}$P<0.001

Significantly greater collagen content (mean±SD) is seen in the HF as compared to control atria and their respective appendages. [LA; left atrial, LAA; left atrial appendage, RA; right atrial, RAA; right atrial appendage]

Chapter Six

Atrial Protective Effects of n-3 Polyunsaturated Fatty Acids: A Long Term Study in Ovine Chronic Heart Failure

6.1 INTRODUCTION

The role of n-3 polyunsaturated fatty acids (n-3 PUFAs) in the prevention of atrial fibrillation (AF) remains unclear as clinical research has shown conflicting results to date.[461-469] This is largely due to the heterogeneity of patient populations or underlying atrial substrates, variable n-3 PUFAs formulations or dosing and different types of AF studied.[470-472] In contrast, the bulk of pre-clinical evidence suggests a beneficial atrial anti-arrhythmic effect even though questions remain regarding its mechanisms of action.[473-476]

Specifically, n-3 PUFAs have been associated with reduced incidence of chronic heart failure (HF) with mortality benefits and fewer HF hospitalizations.[477-480] Whether this association can be extended to HF related AF remains unknown in the clinical setting. Here, we evaluated the effects of long term n3-PUFAs supplementation on atrial remodeling in a recently characterized ovine model of doxorubicin-induced, non-ischemic cardiomyopathy.[446, 447]

6.2 METHODS

A total of twenty one Merino Cross wethers were studied. All procedures were conducted in accordance with the guidelines outlined in the "Position of the American Heart Association on Research Animal Use" adopted on November 11, 1984 by the American Heart Association. Approval for the performance of the study was provided by Animal Ethics Committees of the Institute of Medical and Veterinary Services and the University of Adelaide, Adelaide, Australia.

6.2.1 Study Protocol

All animals (n=21) were acclimatized for ≥1week prior to study commencement. Figure 1 details the timeline of the HF model with sequence of investigative assessments and n-3 PUFAs supplementation. Animals were sequentially allocated to the following groups: control (CTL, n=7), HF with n-3 PUFAs supplementation (HF-PUFA, n=7) and HF controls treated with olive oil (HF-CTL, n=7). HF was induced by repeated intracoronary doxorubicin infusions. This is described below together with the fatty acids supplementation protocol. The control group was not sham operated or catheterized and did not receive any doxorubicin or olive oil supplementation. All animals were fasted for 24-hours prior to general anesthesia for all investigational (echocardiography - TTE and cardiac magnetic resonance imaging - CMR) and interventional procedures (pericardial window, cardiac catheterization and electrophysiological studies).

Intravenous sodium thiopentone (15-20 mg/kg) was used for induction before endo-tracheal intubation, while isoflurane (2-4%) in 100% oxygen was utilized for maintenance. Invasive blood pressure, heart rate, pulse oximetry, end-tidal CO2 and temperature were continuously monitored. Post-operative care (pericardial window) included intra-muscular administration of xylazine hydrochloride and penicillin for 3-days.

6.2.2 Doxorubicin Non-ischemic Cardiomyopathy Model

The establishment and characterization of this model has been described elsewhere.[447] In brief, all animals underwent creation of a small 3cm pericardial window to avoid inflammatory pericardial effusion at baseline prior to cardiac imaging and doxorubicin dosing. Doxorubicin (1 mg/kg) was infused over 30 minutes via catheterization of the left-sided coronary arteries (Amplatz AL1 catheter, Cordis Corp, Miami, FL, USA) under fluoroscopic guidance at fortnightly intervals. A total of 3 to 4 doses were required to achieve at least moderate left ventricular systolic dysfunction. Previous validation study showed no evidence of myocardial infarction with this HF model using delayed enhanced cardiac magnetic resonance scans and histological examination.[447]

6.2.3 n-3 PUFAs Supplementation Protocol

n-3 PUFAs treated animals received 1.8 g of eicosapentaenoic acid (EPA) and 1.2 g of docosahexaenoic acid (DHA) per day. HF-CTL animals received 10ml per day of olive oil supplementation consisted of mainly n-6 and monounsaturated n-9 PUFAs; a common placebo used in n-3 PUFAs trials. Supplementation began 1 week prior and continued for 6 weeks following the last doxorubicin dosing (total of 13-15 weeks). Incorporation of n-3 PUFAs in atrial tissues was determined by gas chromatography as previously described.[481]

6.2.4 Cardiac Functional Assessments

CMR was utilized to assess both left atrial and left ventricular volumes and ejection fraction at baseline and prior to the euthanasia studies (Siemens Sonata 1.5 Tesla & Leonardo workstation, Siemens AG, Munich, Germany) with slice thickness of 6 mm through the atria and 10 mm through the ventricles without any inter-slice gap. Mechanical ventilation was maintained to facilitate ECG-gated image acquisition with adequate breath-holding. TTE was used to monitor cardiac function serially during the doxorubicin dosing protocol (Acuson XP-128, 4 MHz probe, Siemens Medical Systems, PA, USA).

6.2.5 Electrophysiological Study

Open chest electrophysiological studies were performed via bilateral thoracotomy approach whereby physiological arterial blood gases and body temperature were maintained throughout. A custom designed 128-electrode epicardial plaques with 5mm inter-electrode distance were then applied to the RA, LA and traversing the Bachmann's bundle before being attached to a computerized recording system (LabSystem Pro, Bard Electrophysiology, MA, USA). Surface-ECG and overlapping bipolar electrograms were continuously monitored and stored for off-line analysis. Electrograms were filtered from 30-500Hz, and measured with computer-assisted calipers at a sweep speed of 200mm/s.

6.2.5.1 Atrial ERP

Atrial ERP was measured at twice diastolic threshold at cycle lengths (S1) of 500, 400, 300 and 200ms from six sites (right atrial appendage – RAA, right atrial free wall – RAFW, right atrial Bachmann's Bundle – RABB, LAA, LAFW, and LABB). Eight basic (S1) stimuli were followed by a premature (S2) stimulus in 10ms decrement. Atrial ERP was defined as the longest S1-S2 interval not resulting in a propagated response. Each measurement was repeated twice and if the variability was greater than 10ms, two further measurements were taken and the total averaged. ERP rate adaptation was calculated as the difference

between ERP_{500} and ERP_{200}. The coefficient of ERP variation (SD/mean x 100%) was determined in each atrium to assess ERP heterogeneity.

6.2.5.2 Atrial Conduction

Conduction was assessed during stable S1 pacing at cycle lengths of 500, 400, 300 and 200ms from four pre-specified sites at the RAA, RAFW, LAA and LAFW. Activation maps were created using semi-automated custom made software (Nucleus Medical, Adelaide, Australia). Each annotation was manually verified with the local activation time annotated to the peak of the largest amplitude deflection on bipolar electrograms. Local conduction velocity was calculated from the local vectors within each triangle of electrodes.[340, 426] A mean conduction velocity can then be derived for each activation map.

Heterogeneity of Conduction

Conduction heterogeneity was assessed using established phase mapping technique during S1 pacing. In brief, the largest activation time difference between every four adjacent electrodes was first determined and divided by inter-electrode distances. The largest value at each site was then used to create a phase map, with values also displayed as a histogram. Absolute conduction phase delay was calculated by subtracting the 5^{th} from the 95^{th} percentile of the phase-difference distribution (P_{5-95}). The conduction heterogeneity index was then calculated by dividing the absolute phase delay by the median (P_{50}).

6.2.5.3 P-wave Duration

P-wave duration was averaged over ten consecutive beats as a surrogate marker of inter-atrial conduction time and measured on lead II of the surface-ECG.

6.2.5.4 AF Inducibility and Duration

AF induction was assessed with rapid atrial pacing at the RAA and using premature stimuli. Rapid pacing started at 200ms cycle length and was decreased in 5ms intervals until either AF was initiated or there was loss of 1:1 atrial capture. A fixed rate of decrement was used at 5ms per 3s to ensure consistency. This protocol of AF induction was repeated ten times. During ERP testing, induced AF episodes were carefully documented with percentage of inducibility taken as the number of AF episodes over the total number of S1-S2 drivetrains delivered. The overall inducibility was the average of inducibility during both ERP testing and rapid decremental pacing. AF was defined as a rapid irregular atrial rhythm lasting more than 2s.

6.2.6 Structural Analysis

Following electrophysiological studies, both atria/appendages were separated and immersed fixed with 10% formalin and paraffin embedded for subsequent

evaluation. Sections were stained with picrosirius red to demonstrate the extracellular matrix in a masked protocol. Five random stained sections from each individual atrium/appendage of every animal were digitally captured (20 sections/animal) for quantification of collagen using image analysis (Analytical imaging Station, Version 6.0, Imaging Research Inc., St. Catherines, Ontario, Canada).

6.2.7 Statistical Analysis

All continuous variables are reported as mean ± standard deviation and assessed for normality utilizing the Shapiro-Wilk test. Data that were normally distributed were compared using the Student's t-test. Data that were not normally distributed were compared using the Wilcoxon rank-sum tests. ANOVA was used for analysis involving 3 groups with post-hoc Tukey's comparisons. Repeated measures ANOVA was used when data was analyzed across more than two cycle lengths. Statistical significance was established at $p<0.05$. The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

6.3 RESULTS

There was one death in the HF-PUFA group 5 days following the third dose of doxorubicin prior to the endpoint CMR and electrophysiological study. Necropsy

revealed changes consistent with pulmonary congestion. This was not unexpected since this HF model has a reported mortality rate of up to 20%. Another 3 animals (2 HF-CTL and 1 HF-PUFA) demonstrated respiratory difficulty in the last 1–2 weeks prior to euthanasia study. This was treated with small doses of furosemide (subcutaneous, 0.5–1 mg/kg) together with fluid restriction. Minor systemic effects of doxorubicin including temporary wool loss, diarrhea, and temporary decrease in hemoglobin, total white cell, and platelet counts were seen in HF-CTL and HF-PUFA animals.

Detailed animal characteristics of the remaining animals in the three groups (n=7 in CTL and HF-CTL, n=6 in HF-PUFA) are presented in Table 1. Animals were well matched for size at study commencement with no significant difference in body weight seen at euthanasia. The average intracoronary doxorubicin dosing and mean duration of was similar in both HF-CTL and HF-PUFA groups (3.4±0.5 vs. 3.5±0.5 mg/kg respectively; p=NS) resulting in moderate degree of cardiomyopathy as assessed by CMR. Likewise, the mean duration of n3-PUFAs or olive oil supplementation did not differ between HF-CTL and HF-PUFA animals (13.6±0.6 vs.13.7±0.6 weeks respectively, p=NS) Pulmonary capillary wedge pressure and heart rate were comparable in all groups. Incorporation of n-3 PUFAs in atrial tissues was evident with 2-3 fold increase in DHA and EPA levels in the HF-PUFA group ($p<0.001$).

6.3.1 n-3 PUFAs and Cardiac Functional Remodeling

n3-PUFAs treatment prevented LA dilatation due to doxorubicin cardiomyopathy with HF-PUFA group demonstrating preserved LA volume as assessed by CMR (Table 1). Although LA ejection fraction was not as impaired in HF-PUFA in comparison to HF-CTL group, this did not reach statistical significance. In contrast, PUFA treatment did not appear to have any effect on changes in left ventricular dimension or function associated with HF.

6.3.2 n3-PUFAs and Atrial Electrical Remodeling

6.3.2.1 Atrial Refractoriness

Treatment with n3-PUFAs resulted in lower ERP in both atria as compared to the HF-CTL and CTL groups (Overall mean ERP averaged across all sites - RA: 134±21 vs. 184±40 vs. 171±34ms; LA: 116±19 vs. 155±35 vs. 136±19ms, respectively at S1=300ms, both $p<0.001$). Figure 1A demonstrates the differences amongst the three groups at different cycle lengths. This difference in ERP was also observed when analyzed by pacing locations (Figure 1B). Physiological rate adaptation did not differ in the CTL versus HF-CTL and HF-PUFA groups (RA: 23±13 vs. 20±16 vs. 13±9ms respectively, p=0.08; LA: 22±22 vs. 16±17 vs. 14±7ms respectively, p=0.3). Likewise, ERP heterogeneity was similar in the CTL versus HF-CTL and HF-PUFA groups (RA: 15±8 vs. 12±8 vs. 14±6%, p=0.7; LA: 11±4 vs. 13±10 vs. 12±8%, p=NS; respectively with S1 at 300ms).

6.3.2.2 Atrial Conduction

n3-PUFAs supplementation attenuated the conduction slowing due to doxorubicin HF with resultant conduction velocity in HF-PUFA group similar to CTL as compared to HF-CTL (Overall mean conduction velocity averaged across all S1 and sites - RA: 0.91±0.11 vs. 0.87±0.16 vs. 0.76±0.09m/s, LA: 0.86±0.12 vs. 0.93±0.15 vs. 0.75±0.09m/s, respectively) at all S1 tested ($p<0.001$, Figure 3A) and when analyzed according to pacing sites (Figure 3B, left panel). Phase mapping showed an increased absolute range of conduction phase delay in HF-CTL as compared to CTL which was significantly reduced in the HF-PUFA group (Overall mean P_{5-95} averaged across all S1 and sites – RA: 2.03±0.93 vs. 1.61±0.49ms/mm vs. 1.61±0.48; $p=0.02$, LA: 2.53±0.77 vs. 1.58±0.42 vs. 1.76±1.23ms/mm, $p<0.001$). Likewise, n3-PUFAs treatment resulted in improved conduction heterogeneity index in HF-PUFA animals to levels comparable to the CTL as compared to HF-CTL (Figure 3B, right panel).

Representative activation maps and corresponding phase histograms during S1 pacing at 300ms from the LAA are shown in Figure 4. Increased isochronal crowding was evident in the HF-CTL as compared to CTL atrium representing slower conduction with longer total activation time of 53 versus 41ms. Significant improvement was noted in the HF-PUFA example whereby the total activation time was only 40ms. In addition, the corresponding phase histograms

illustrated that n3-PUFAs treatment prevented the development of increased conduction heterogeneity seen with HF-CTL.

6.3.2.3 P wave Duration

Prolongation of P wave duration as a result of HF was attenuated with n-3 PUFAs supplementation (CTL: 58±6 vs. HF-CTL: 68±5 vs. HF-PUFA: 61±1ms, p=0.01).

6.3.2.4 AF Inducibility and Duration

No significant difference in AF inducibility was seen amongst the three groups even though the CTL and HF-PUFA groups appeared less susceptible to induced AF than the HF-CTL animals (3±3 vs. 5±4 vs. 22±28s respectively; p=0.2). However, the duration of induced AF episodes were significantly shorter in the HF-PUFA and CTL animals as compared to HF-CTL group (1±1 vs. 2±4 vs. 20±23s; p=0.02).

6.3.3 n3-PUFAs and Atrial Structural Remodeling

Significant difference in the amount of overall interstitial collagen deposition was observed amongst the 3 groups: 4.9±2.6% in HF-CTL vs. 3.9±1.7% in HF-PUFA vs. 2.6±0.9% in CTL (p<0.001). n-3 PUFAs supplementation resulted in a

20% reduction in atrial interstitial fibrosis ($p<0.05$) due to HF. Representative picrosirius red stains from the 3 groups are shown in Figure 5.

6.4 DISCUSSIONS

Long term supplementation with n-3 PUFAs in this ovine model of doxorubicin-induced non-ischemic cardiomyopathy showed significant n-3 PUFAs incorporation in atrial tissues which was protective against the following adverse HF related atrial remodeling:

1. Structural abnormalities characterized by left atrial dilatation and interstitial fibrosis;
2. Conduction abnormalities including conduction slowing with increased heterogeneity and prolonged P wave duration;
3. Prolonged duration of induced AF episodes.

This was despite significantly lower atrial refractoriness, persistent left ventricular dysfunction with increased left atrial filling pressure and similar heart rate to HF-CTL animals. These findings suggest that the development of the substrate predisposing to AF may be attenuated by the use of n3-PUFAs.

6.4.1 Lessons from Existing Studies: Importance of Underlying Substrate

The relationship between fish or n3-PUFAs consumption and risk of AF has been explored by investigators in different patient populations resulting in conflicting conclusions.[461-468, 478] Since the initial positive report regarding fish intake and its association with lower risk of AF from the Cardiovascular Health Study, subsequent reports from the Rotterdam study, Danish Diet, Cancer and Health Study and Women's Health Initiatives have shown no such benefits.[461-463, 468] Virtanen and colleagues further extended this controversy from the above-mentioned studies using dietary fish intake assessments by showing a positive association between serum n-3 PUFAs levels and lower risk of AF.[464] However, in the settings of post-operative AF and in post myocardial infarction patients, n3-PUFAs use has shown more promising results.[465-467] This may be accounted for due to the heterogeneity of the populations studied in terms of age, concurrent cardiovascular disease or risk profile, dietary composition and lifestyle factors.[471, 472] Therefore, more studies are warranted in determining the anti-arrhythmic role of n-3 PUFAs supplementation in patients with different underlying AF substrate.

6.4.2 n3-PUFAs in Heart Failure

Although n3-PUFAs have been shown to reduce the risk of HF and HF related mortality and hospitalizations, no clinical studies have addressed its potential role in HF related AF.[477-480] Nevertheless, limited insights could be gained from experimental work reported by Sakabe and colleagues.[473] Using a canine rapid ventricular pacing model of HF, this short term study demonstrated that n-3 PUFAs attenuated atrial fibrosis, conduction heterogeneity and AF promotion. However, the following concerns remain: First, rapid ventricular pacing induced HF may not be representative of all types of cardiomyopathy in the HF syndrome. Second, atrial remodeling due to rapid pacing rate and the resultant ventricular dyssynchrony cannot be excluded.[450] The rapid pacing HF model also precluded the assessment of n-3 PUFAs effects on heart rate and left ventricular function. Third, the above findings were observed together with improved left ventricular hemodynamics which may have confounded the true atrial effects of n-3 PUFAs. Fourth, atrial incorporation of n-3 PUFAs was not demonstrated with the high dose supplementation regimen (5.28g/day) over a short 4-week period. Finally, atrial size and function were not evaluated. Due to the above limitations, further study of this important AF substrate in a different animal model of HF is therefore mandated.

The clinical entity of non-ischemic cardiomyopathy encompasses a wide range of etiology which includes exposure to cardiotoxic agent such as doxorubicin.

Recent work using intra-coronary delivery of doxorubicin has resulted in good animal tolerability and survival with demonstrable changes representative of non-ischemic cardiomyopathy seen in humans.[446, 447] This novel model of doxorubicin-induced HF has been shown to produce comparable HF related atrial remodeling and was therefore chosen for this study to provide further evidence on the anti-arrhythmic effects of n3-PUFAs in the HF atria.[482] Importantly, the present study demonstrated that atrial incorporation of n-3 PUFAs from long term supplementation (3g/day) prevented adverse atrial remodeling independent of hemodynamic factors and left ventricular functional change in a more physiological model of HF. It complements existing literature by affirming the atrial protective role of n-3 PUFAs in HF. Here, we also demonstrated for the first time that long term n-3 PUFAs prevented LA dilatation due to HF together with slight improvement in LA function. Indeed, LA enlargement has been associated with AF whereby every 5mm increase in LA size will result in 39% increased risk of developing AF.[271, 327] Likewise, greater LA diameter reduction following treatment has been associated with lower incidence of new onset AF.[483]

6.4.3 Atrial Electrophysiology and n-3 PUFAs

The anti-arrhythmic mechanisms of n-3 PUFAs are likely to be multi-dimensional with anti-inflammatory, anti-fibrotic, anti-oxidant, anti-sympathetic and pro-autonomic properties in addition to various atrial electrophysiological effects.[484]

These anti-fibrillatory electrophysiological mechanisms include: Inhibitory effects on the transient outward and ultra-rapid delayed rectifier potassium currents (Ito and IKur) and the voltage-gated sodium current (INa) as shown in human atrial myocytes [485]; Modulation of atrial gap junction proteins (connexins 40) seen in canine atria [476]; Anti-asynchronous contractile effects due to increased membrane fluidity demonstrated in rat atrial myocytes[486]; and improved conduction with reduced heterogeneity in canine atria from two different pacing models.[473, 474] These beneficial effects of n3-PUFAs on atrial conduction properties were also evident in our HF-PUFA sheep.

Previous studies have shown no changes in atrial refractoriness with n-3 PUFAs supplementation in a simultaneous atrioventricular pacing and the rapid ventricular pacing canine studies.[473, 474] In Langendorff-perfused rabbit hearts, n-3 PUFAs supplemented group had significantly longer atrial refractoriness which were also less susceptible to stretch related decrease in ERP seen in the control group.[475] Also, acute n-3 PUFAs infusion has been shown to prevent abbreviation of ERP with rapid atrial pacing which was not seen with oral supplementation.[473, 487] In contrast, our HF-PUFA sheep showed reduced bi-atrial refractoriness. This could be secondary to different n-3 PUFAs formulations and treatment duration in each study, or variation due to different animal model. Traditionally, changes in atrial ERP in different established animal models have been variable and underlying conduction abnormalities as a result

of structural remodeling were considered more important in atrial arrhythmogenesis.[200, 363, 435, 474, 482, 488] Nevertheless, with the attenuation of HF related conduction abnormalities, our HF-PUFA animals had shorter induced AF episodes than HF-CTL despite lower ERP.

6.4.4 Clinical Significance

By affirming the atrial protective role of n-3 PUFAs in different HF models, this work provides further impetus to study its effects in patients with HF and AF. In light of the emerging dual epidemics of AF and HF, n3-PUFAs may potentially provide a relatively affordable and non-toxic option to prevent adverse atrial remodeling and reduce AF burden in this subgroup of patients.[3, 352] In particular, n-3 PUFAs have been shown to provide additional albeit modest improvement in clinical outcomes of HF patients above current evidence-based therapies.[489] Several studies have demonstrated marked structural change and its electrophysiological manifestations as the substrate predisposing to AF. [169, 200, 302-304, 363, 377, 435, 482, 488] Recently it has been suggested that this substrate may be reversible with treatment of the primary condition.[490] The current study provides evidence to suggest that the substrate for AF in HF may be prevented with the use of n-3 PUFAs. However, whether n-3 PUFAs prevents the substrate for AF in other conditions or if other disease modifying agents could have a similar role is yet to be determined.

6.4.5 Study Limitations

Direct extrapolation of findings in this animal model to human HF is cautioned although the similarity of our findings to that from rapid ventricular pacing model implies its validity. Electrophysiological study at different time-points would have provided incremental information regarding reverse remodeling effects due to n-3 PUFAs. Likewise, additional groupings with different dosing or formulation of n-3 PUFAs would provide further useful information.

6.5 CONCLUSIONS

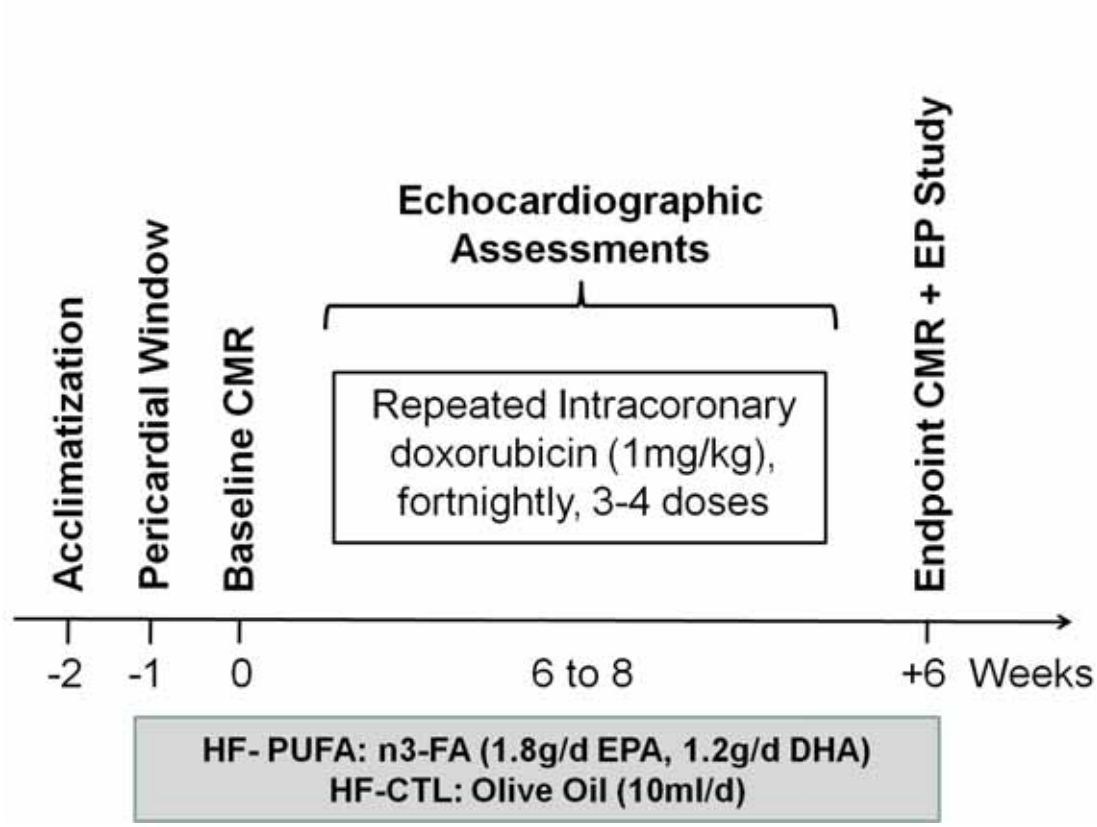
This study provides further evidence that n-3 PUFAs can prevent the development of the AF substrate of HF.

Table 1: Animal Characteristics

	CTL (n=7)	HF-CTL (n=7)	HF-PUFA (n=6)	P
Weight, baseline (kg)	48±6	51±7	47±8	0.5
Weight, euthanasia (kg)	49±6	53±6	48±7	0.4
Heart rate (beats/min)	108±15	106±9	99±8	0.5
PCWP (mmHg)	10±2	10±4	9±2	0.7
Atrial DHA (%)	2.1±0.2	1.8±0.5	4.3±0.1‡*	<0.001
Atrial EPA (%)	2.9±0.6	3.5±0.5	9.1±1.4‡*	<0.001
CMR				
LA EDV (ml)	29±7	40±2†	28±2*	0.001
LA ESV (ml)	19±2	33±5†	21±2*	<0.001
LA EF (%)	32±2	25±5†	29±3	0.008
LV EDV (ml)	76±3	73±6	75±5	0.6
LV ESV(ml)	40±6	49±9	51±9	0.1
LV EF (%)	44±7	36±5†	35±6‡	0.004

Post-hoc comparisons with p<0.05: † HF-CTL vs. CTL; ‡ HF-PUFA vs. CTL; *HF-PUFA vs. HF-CTL. PCWP, pulmonary capillary wedge pressure; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LV, left ventricular; LA, left atrial; EDV, end diastolic volume; ESV, end systolic volume; EF, ejection fraction.

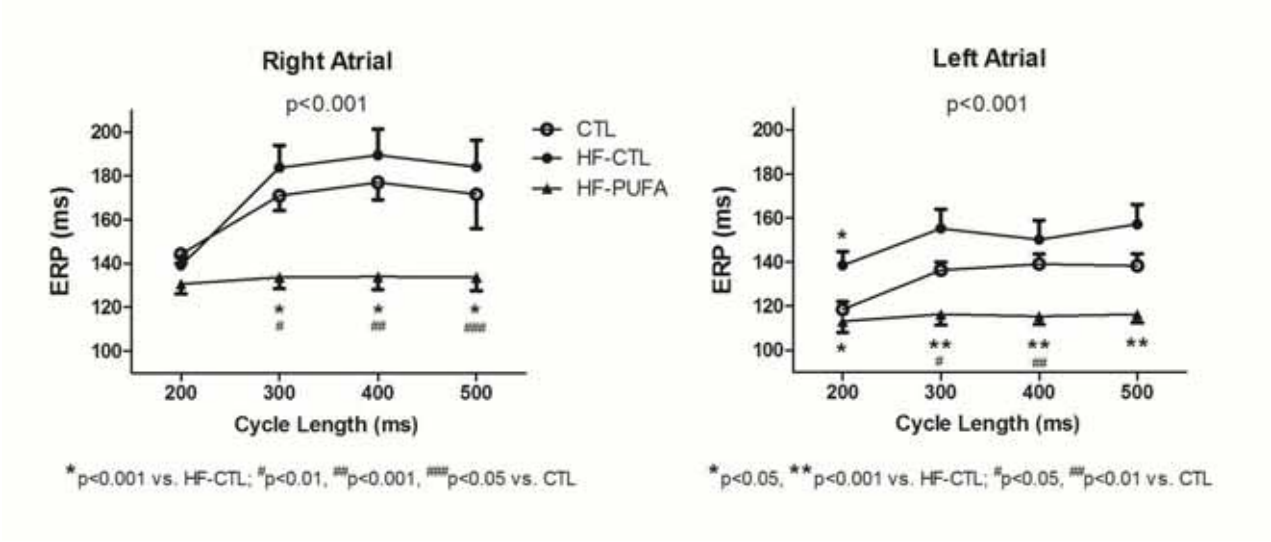
Figure 1: Model Timeline



This figure illustrates the time sequence of interventions, supplementations and investigations from acclimatization to endpoint electrophysiological study.

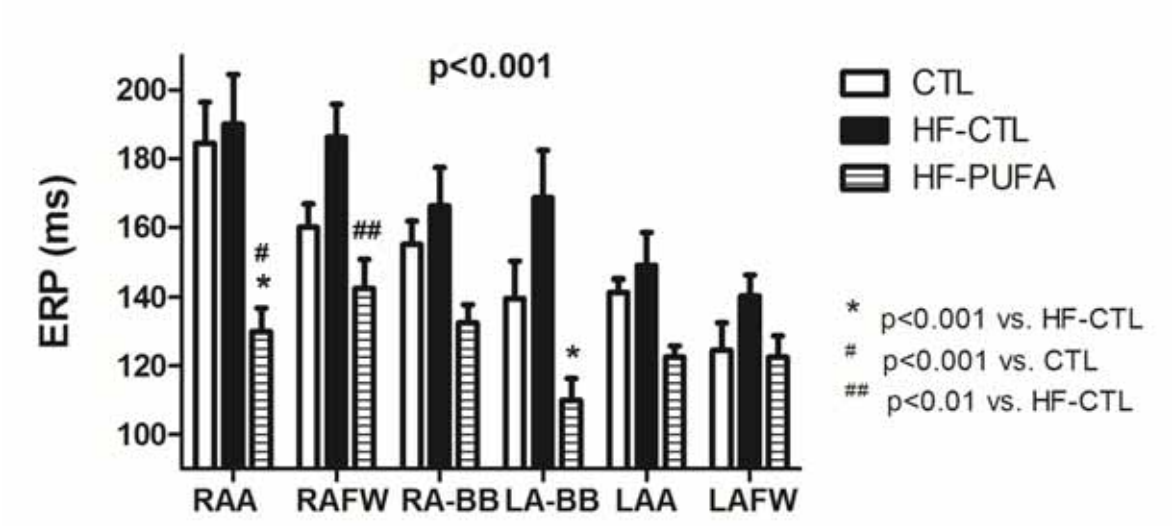
Figure 2: Atrial ERP

A Mean ERP at different pacing cycle lengths



ERP (mean±SEM) was uniformly lower in the HF-PUFA animals at all pacing cycle lengths compared to CTL and HF-CTL. This graph utilized pooled data from all pacing sites of the respective atrium.

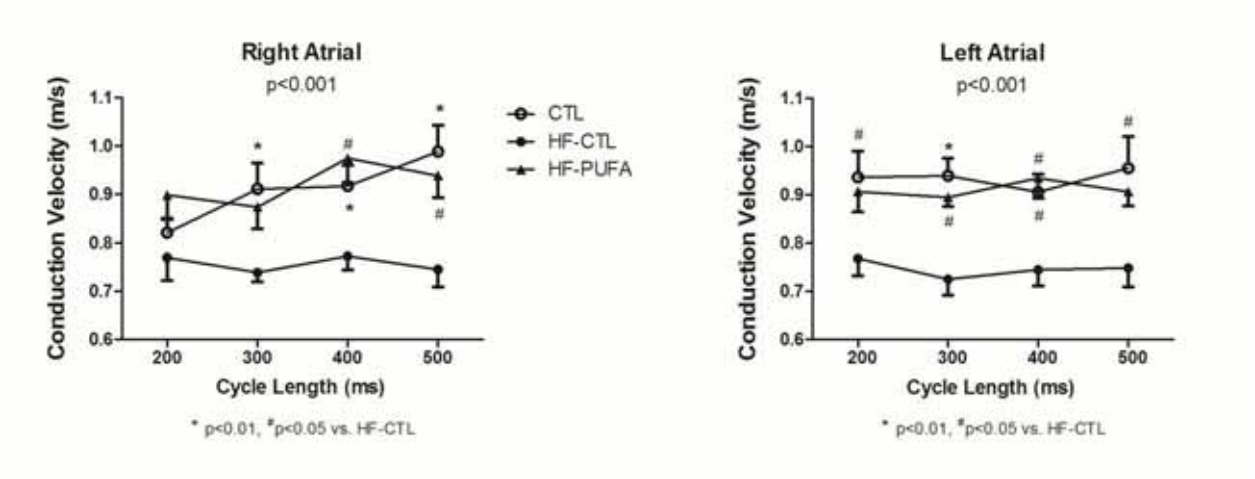
B Mean ERP by pacing locations (S1 = 300ms)



Similarly, when analyzed by pacing sites, ERP (mean±SEM) was lower in the HF-PUFA group. This graph illustrates the difference amongst groups during pacing cycle length of 300ms.

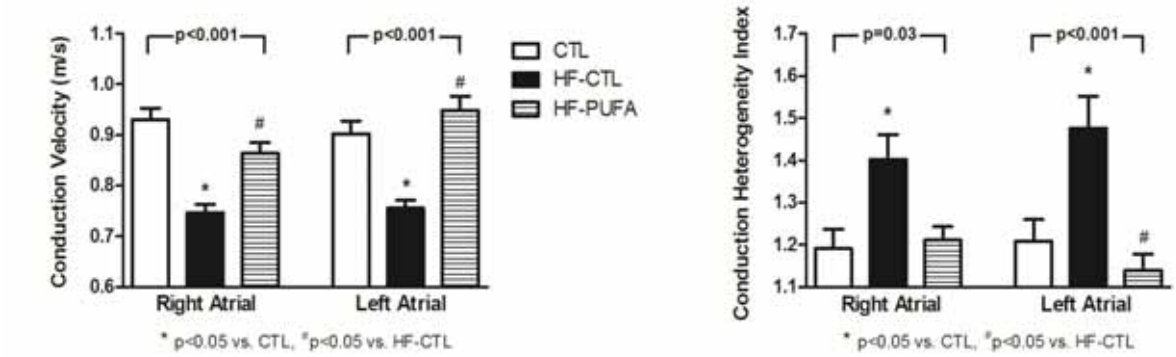
Figure 3: Atrial Conduction

A Mean Conduction Velocity at different pacing cycle lengths



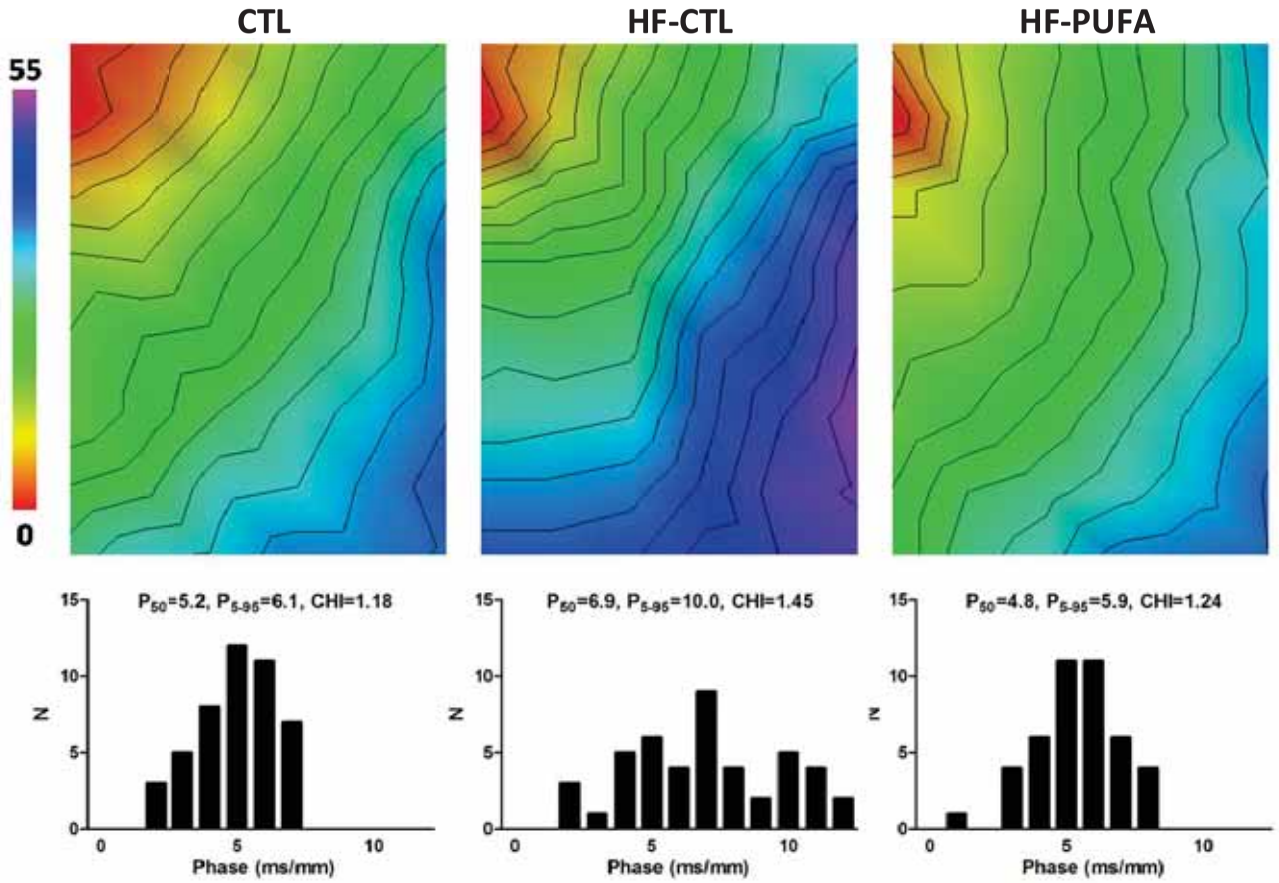
HF resulted in slower conduction velocity (mean±SEM) at all pacing cycle lengths in both atria of the HF-CTL group as compared to CTL. This conduction slowing was prevented by n3-PUFAs supplementation. This graph utilized pooled data from all pacing sites of the respective atrium.

B Conduction Velocity and Heterogeneity Index by Atrial



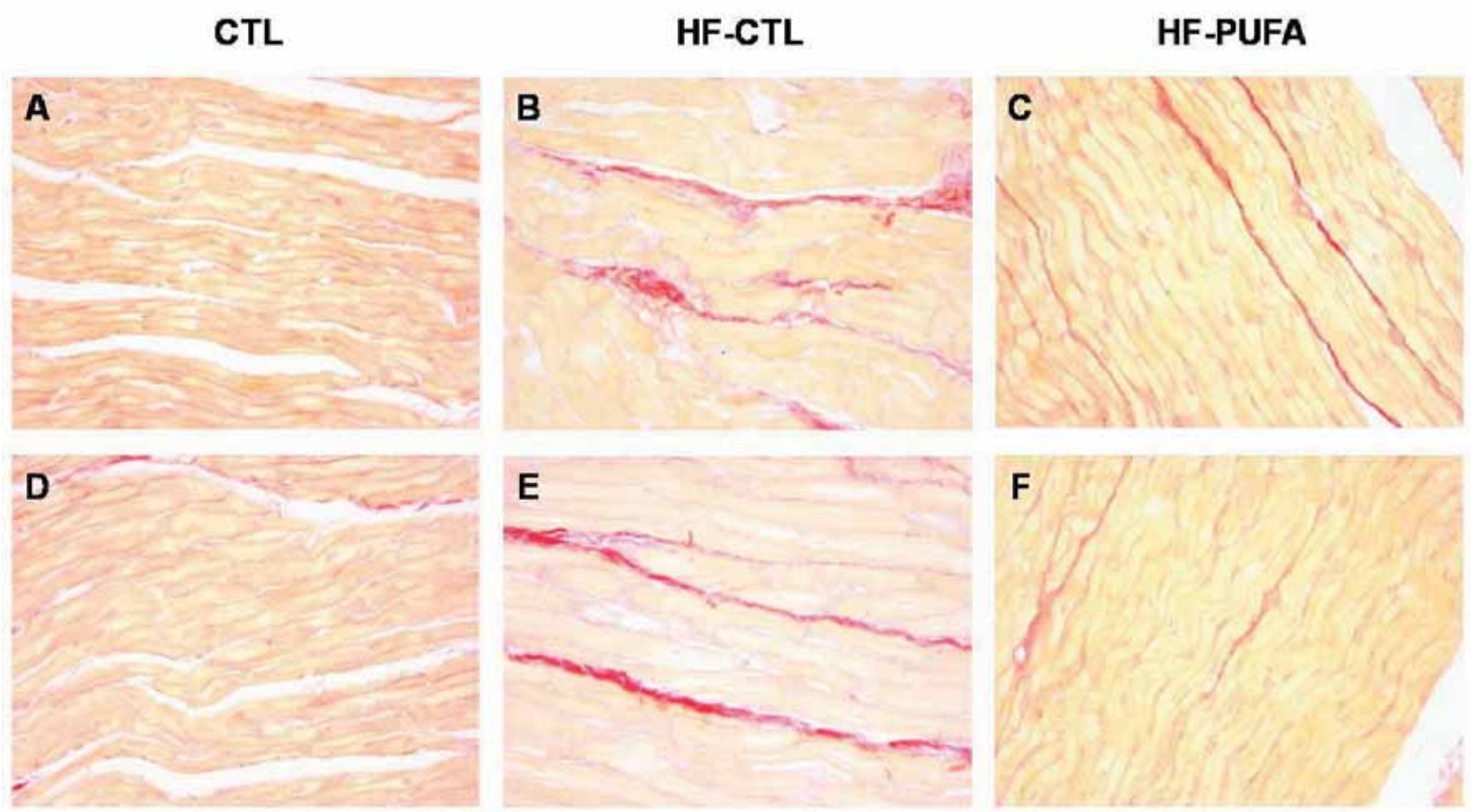
Similar differences in conduction velocity were also observed when analyzed according to pacing site (left panel). Significant effect of n3-PUFAs on conduction heterogeneity index was only seen in the left atrium with a positive trend seen with the data from the right atrium (right panel).

Figure 4: Activation Maps and Phase Histograms



Representative activation maps (top panel) and corresponding phase histograms (bottom panel) during S1 pacing at 300ms from the LAA are shown here. Significant isochronal crowding in the HF-CTL example where there was increased conduction heterogeneity was not seen with n3-PUFAs treatment. In fact, activation maps and phase histograms of the CTL and HF-PUFA appear similar suggestive of a positive protective role of n3-PUFAs against atrial conduction abnormalities due to HF.

Figure 5: Representative Picrosirius Red Stains



Representative picrosirius red stains from the CTL, HF-CTL and HF-PUFA LA (top panel: A-C) and RA (bottom panel: D-F) are shown here (magnification x350). Significant increase in interstitial fibrosis is evident in HF-CTL as compared to CTL atria. Reduced amount of interstitial collagen deposition is seen in the HF-PUFA atria.

Chapter Seven

Final Discussions

This thesis presents detailed evaluation of atrial remodeling in animal models of hypertension and heart failure, which are both important risk factors for atrial fibrillation. It provides novel insights into these substrates predisposing to the development of atrial fibrillation with focus on the time course of remodeling in hypertension and the atrial effects of omega-3 fatty acids in heart failure. The consistent findings of atrial interstitial fibrosis and its resultant conduction abnormalities in different substrates for atrial fibrillation highlighted the importance of structural remodeling leading to the perpetuation of this arrhythmia. Despite what appears to be 'remodeling of the same sort' resulting in increased atrial fibrosis, the precise mechanisms of atrial fibrillation in these conditions remain incompletely understood. The variable changes in atrial refractoriness seen in ovine hypertension and heart failure models, as well as following overall beneficial effects of omega-3 fatty acids supplementation, have strengthened the case for structural remodeling contributing to atrial fibrillation.

Detailed characterization of cardiac remodeling in large animal models of hypertension is presented in Chapter 2. The cardiac changes in this 'one-kidney, one-clip' hypertensive model are representative of human hypertensive heart

disease. Therefore, this large animal model will be applicable to cardiovascular research related to high blood pressure. The effects of short term hypertension on atrial remodeling have not been previously evaluated. In Chapter 3, significant atrial remodeling characterized by atrial enlargement/dysfunction, interstitial fibrosis, inflammation, slowed/heterogeneous conduction, increased atrial refractoriness and a greater propensity for atrial fibrillation is evident even at 7 weeks of hypertension. This finding is clinically important as it provides an impetus for early aggressive treatment of hypertension for the prevention of atrial fibrillation.

Chapter 4 presents the time course of atrial remodeling in chronic hypertension using conscious chronically instrument hypertensive sheep. Not surprisingly, hypertension is associated with early and progressive changes in atrial remodeling. Atrial remodeling occurs at different time domains in chronic hypertension: Progressive bi-atrial hypertrophy, left atrial dysfunction and greater atrial fibrillation inducibility are seen early (within 5 weeks) with increased atrial inflammation; Significant conduction slowing with increased heterogeneity and interstitial fibrosis are seen later (from 10 weeks) resulting in more fractionated and longer atrial fibrillation episodes. In addition, there is significant electro-structural correlate in this remodeling cascade. This study supports the importance of treating hypertension in a timely manner before remodeling progresses to an unfavorable milieu capable of sustaining longer

atrial fibrillation episodes. In addition, both Chapter 3 and 4 highlight that fibrosis and inflammation may be novel targets for more atrial selective therapies in the hypertensive patients with atrial fibrillation.

To date, all pre-clinical studies of atrial remodeling in heart failure have been confined to a single model of rapid ventricular pacing. Chapter 5 evaluates atrial remodeling in a recently characterized ovine model of non-reversible, doxorubicin-induced non-ischemic cardiomyopathy. The changes of atrial enlargement/dysfunction, increased fibrosis, slowed/ heterogeneous conduction and increased refractoriness led to more sustained atrial fibrillation in this model of heart failure. These findings appear the “same sort” to previous models of heart failure implicating a final common substrate leading to the development of atrial fibrillation in this condition. Nevertheless, this novel model has the advantages of producing a more permanent non-reversible heart failure with better suitability for interventional studies examining reverse remodeling and is more compatible to state of the art cardiac magnetic resonance imaging.

The role of omega-3 fatty acids in the prevention of atrial fibrillation remains unclear as clinical research has shown conflicting evidence to date. Clinically, omega-3 fatty acids have been shown to provide additional albeit modest improvement in outcomes of heart failure patients above current evidence-

based therapies. Chapter 6 examines the role of omega-3 fatty acids in atrial fibrillation due to heart failure. In this ovine heart failure model, chronic omega-3 fatty acids use is protective against adverse atrial remodeling by preventing atrial enlargement, fibrosis and conduction abnormalities leading to shorter atrial fibrillation episodes. This work provides further impetus to study the effects of omega-3 fatty acids in patients with heart failure and atrial fibrillation. Potentially, this may provide a relatively affordable and non-toxic option to prevent adverse atrial remodeling and reduce atrial fibrillation burden in heart failure patients.

Chapter Eight

Future Directions

Improved understanding of the mechanisms of atrial fibrillation in different substrates is crucial in our effort to treat this complex arrhythmia. Structural changes of atrial fibrosis resulting in conduction abnormalities have been repetitively demonstrated in different atrial substrates. Preventing or reversing structural changes should be a therapeutic goal. It is likely that atrial-specific anti-fibrotic agents or cell based gene therapy will be studied in the near future. However, the pathways leading to atrial fibrosis remain poorly elucidated and in-vivo quantification of atrial fibrosis is still in its infancy. Recent work has shown that the substrate of atrial fibrillation is reversible when the underlying factor is treated.[490] Therefore, targeting of atrial fibrillation risk factors is of paramount importance.

In facing the emerging epidemic, primary and secondary prevention of atrial fibrillation will be required to reduce its economic burden on healthcare systems worldwide. Current guidelines in the management of atrial fibrillation do not provide an optimal blood pressure treatment target. Specifically, there is emerging evidence that pre-hypertension is a strong and independent predictor of incident atrial fibrillation. Perhaps future studies will provide further

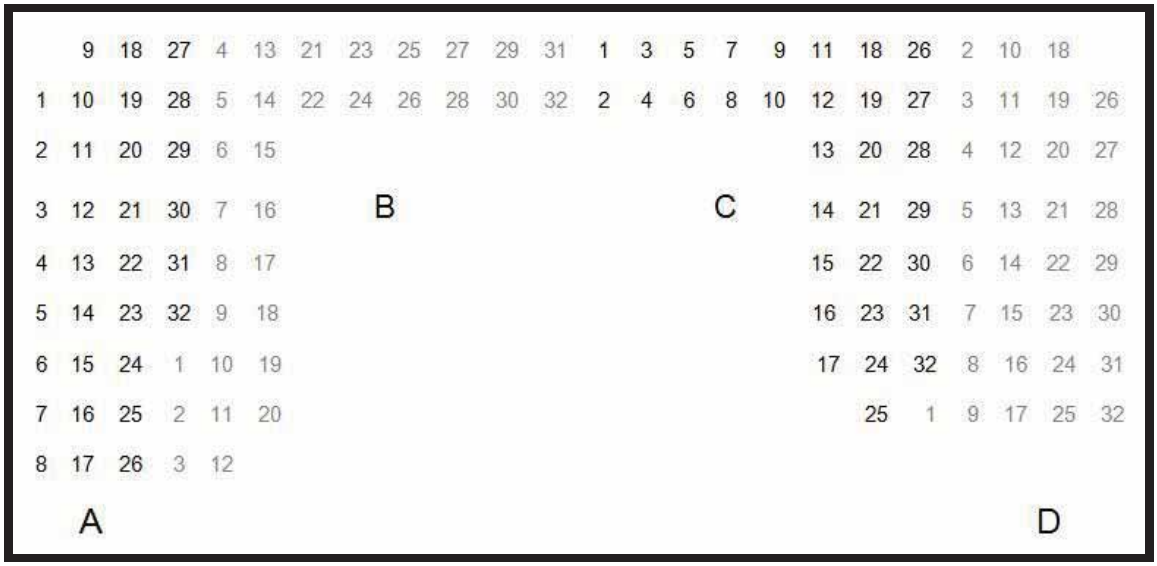
guidance on upstream therapies of conditions such as hypertension and heart failure as primary prevention of atrial fibrillation.

In the absence of a curative therapy, the management of AF has been limited to stroke prevention, rate control or maintenance of sinus rhythm. Pharmacological management of AF has been limited by the non-availability of atrial-specific anti-arrhythmic agents and the significant side effects profile of current available drugs. Although catheter ablation therapy for atrial fibrillation is likely to play an increasing role in our combat of this arrhythmia, the variable techniques adopted in different centers highlight the current knowledge gap in the underlying mechanisms of this complex arrhythmia.

APPENDIX 1

Custom Designed Epicardial Plaque

Electrode Spacing & Numbering



The plaque was made of silicone with 128 pure silver electrodes spaced 5mm apart equally in all directions. They were grouped into four separate circular connectors (A-D) containing 32 electrodes each, for connection to the electrophysiology recording system. Sequential bi-poles were used whenever possible to maximize the density of recording (i.e. A1-2, A2-3, A3-4…, A9-10, A10-11, A11-12…) Pacing was performed from six pre-specified sites (A1, A8, B21, C9, D26 & D32)

Chapter Nine

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