ACUTE AND CHRONIC ATRIAL REMODELING IN OBSTRUCTIVE SLEEP APNOEA: IMPLICATIONS FOR ATRIAL FIBRILLATION

By

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Dedicated to
My Mother
My Father
&
My wife Rhiannon
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ABSTRACT

Atrial fibrillation (AF), the most common sustained arrhythmia, has been well studied; however, its underlying mechanisms and relationships to other disease processes have not been fully explored. Observational data from epidemiological studies have suggested a relationship between obstructive sleep apnoea (OSA) and AF. Recent clinical studies have implicated an adverse outcome to therapy in patients with AF and OSA. Despite several candidate mechanisms advanced, the acute and chronic changes to the atrial myocardium have not been fully characterised.

This thesis evaluates symptomatic patients with AF presenting for radiofrequency ablation of their arrhythmia. The acute and chronic electrophysiological and electroanatomical atrial substrate is characterised. Finally the effect on clinical outcomes of therapy directed at AF is evaluated with specific reference to the presence of OSA.

Chapter 2 demonstrates the differences in the atrial substrate in paroxysmal and persistent AF. This study found persistent AF was associated with a reduction in electrogram voltage and greater signal fragmentation, with these two attributes being negatively associated.

Chapter 3 examines patients presenting with symptomatic AF, previously not known to have sleep disordered breathing. OSA was associated with a greater symptomatic burden of AF as well as chronicity of the arrhythmia. In the presence of more severe OSA, there was a greater chance of failure of radiofrequency ablation in maintaining sinus rhythm.
Chapter 4 characterises the underlying atrial substrate resulting in AF in patients with moderate to severe untreated OSA. OSA patients had larger atria, greater areas of low voltage and areas of electrical silence, suggesting loss of atrial myocardium. There were also markedly reduced conduction velocities, longer corrected sinus node recovery times and more conduction abnormalities characterised by complex electrograms. These findings provide important insights into the adverse remodeling that may allow AF to develop and persist in these patients, and promote the failure of ablation strategies.

Chapter 5 examines nocturnal atrial electrophysiological alterations resulting from acute episodes of respiratory disturbance associated with hypopnoea and obstructive apnoea events the night after undergoing radiofrequency ablation for AF. Dynamic changes in effective refractory period (ERP), conduction times and conduction delay along linear catheters were documented. The changes appeared to be more marked for obstructive apnoeas than hypopnoeas. This study suggests a dynamic, pro-arrhythmic electrical substrate that could potentially lead to nocturnal triggering of AF and its maintenance.

Finally, Chapter 6 describes an ex-vivo rabbit model, created to examine the acute effects of hypoxia (moderate and severe) and hypercapnia. Left atrial ERP, conduction time and conduction heterogeneity were studied using a customised microelectrode array. During hypoxia, there was a dose dependent increase in ERP with only partial resolution on restoration of baseline oxygen levels. With hypercapnia, there was a slower rise in ERP that did not appear to recover. Slowing of conduction was most noted in severe hypoxia, with
only partial resolution in recovery. In hypercapnia, there was progressive slowing of conduction into recovery. Conduction heterogeneity was also increased in the presence of hypoxia and hypercapnia. These findings further suggest complex alterations to the atrial electrophysiology in the presence of hypoxia and hypercapnia, with dynamic changes to in refractory periods, conduction times and heterogeneity.

Together, a greater understanding of the acute and chronic effects of OSA on AF, and an appreciation of the failure of ablation strategies in the presence of this breathing disorder is attained. This paves the way for further studies on the mechanisms involved, and on the potential role for continuous positive airway pressure therapy (CPAP) in the management of patients with OSA and AF.
DECLARATION

This work was performed by the candidate at the Centre for Heart Rhythm Disorders at the University of Adelaide and Royal Adelaide Hospital, South Australia (during the years 2007 through 2011).

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 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.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works derived from this thesis (specifically, chapter 4) resides with the copyright holder of those works.


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CHAPTER 3:
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Hany Dimitri, Michelle Ng, Anthony Brooks, Andrew Thornton, Hany Abed, Muayad Alasady, Dennis Lau, Han Lim, Doug McEvoy, Ral Antic, Prashanthan Sanders. The severity of obstructive sleep apnoea determines the persistence and severity of atrial fibrillation. Heart, Lung and Circ.; 19: Supplement 2, S114

ABSTRACT POSTER PRESENTATION: HEART RHYTHM SOCIETY SCIENTIFIC SESSIONS 2010

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ABSTRACT POSTER PRESENTATION:
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ORAL PRESENTATION:
HEART RHYTHM SOCIETY SCIENTIFIC SESSIONS 2010
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AF</td>
<td>atrial fibrillation</td>
</tr>
<tr>
<td>AHI</td>
<td>apnoea hypopnoea index</td>
</tr>
<tr>
<td>CFAE</td>
<td>complex fractionated atrial electrograms</td>
</tr>
<tr>
<td>CPAP</td>
<td>continuous positive airway pressure</td>
</tr>
<tr>
<td>CSA</td>
<td>central sleep apnoea</td>
</tr>
<tr>
<td>CSNRT</td>
<td>corrected sinus node recovery time</td>
</tr>
<tr>
<td>ERP</td>
<td>effective refractory period</td>
</tr>
<tr>
<td>HR</td>
<td>hazard ratio</td>
</tr>
<tr>
<td>IQR</td>
<td>interquartile range</td>
</tr>
<tr>
<td>LA</td>
<td>left atrium</td>
</tr>
<tr>
<td>LV</td>
<td>left ventricle</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>OSA</td>
<td>obstructive sleep apnoea</td>
</tr>
<tr>
<td>RA</td>
<td>right atrium</td>
</tr>
<tr>
<td>RDI</td>
<td>respiratory disturbance index</td>
</tr>
<tr>
<td>RR</td>
<td>relative risk</td>
</tr>
<tr>
<td>RV</td>
<td>right ventricle</td>
</tr>
<tr>
<td>SDB</td>
<td>sleep disordered breathing</td>
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INTRODUCTION & LITERATURE REVIEW

1.1 INTRODUCTION

Atrial fibrillation (AF), a ‘supraventricular tachyarrhythmia characterised by uncoordinated atrial activation’,¹ is the most common sustained cardiac rhythm disturbance. Symptoms such as palpitations, fatigue, dyspnoea, heart failure and thromboembolism resulting from this arrhythmia cause significant morbidity and mortality, culminating in substantial health care cost.¹ Data from the United States suggests that by the year 2050 over 12 million people will have AF.²³ Australian data suggests that this condition has rapidly become one of the common cardiovascular causes for hospitalisation; currently ahead of heart failure.⁴ Therefore this condition is poised to present a significant cost to the health system and the community.

Mechanistically, AF requires an initiator or trigger (such as from the atrial sleeves in the proximal pulmonary veins), as demonstrated by Haissaguerre et al. over a decade ago,⁵ as well as an underlying substrate to allow perpetuation of the arrhythmia. An adversely remodelled atrial myocardial substrate associated with AF has been demonstrated in various disease states including congestive cardiac failure,⁶ sinus node disease,⁷ atrial septal defect⁸ and mitral valve disease.⁹ Historically, atrial fibrillation in the absence of age >60, echocardiographic or clinical cardiopulmonary disease including hypertension, has been referred to as ‘lone’ AF.¹ However, recently, Stiles et al. demonstrated that even lone AF patients, remote from any arrhythmia occurrence, demonstrate subclinical biatrial modification of
the myocardium, indicating the presence of an unidentified ‘second factor’ predisposing to the development and progression of AF.\textsuperscript{10}

Sleep disordered breathing includes both obstructive sleep apnoea (OSA) characterised by interruption of ventilation during sleep secondary to pharyngeal collapse, and central sleep apnoea (CSA) characterised by repetitive cessation of ventilation due to loss of ventilatory drive, and occurring alone (idiopathic) or in the presence of heart failure such as Cheyne-Stokes respiration.\textsuperscript{11} In OSA, repetitive apnoeas wither from cessation of breathing or excessive respiratory movements against a collapsed airway during sleep, results in intermittent hypoxaemia and hypercapnia, and culminates in surges of sympathetic neural activity that have profound and long term effects.\textsuperscript{11,12} These include hypertension,\textsuperscript{13} metabolic dysregulation,\textsuperscript{14} stroke,\textsuperscript{15} defects in neurocongnition\textsuperscript{16} and increased risk of motor vehicle accidents.\textsuperscript{17} In particular, not only do OSA patients have features in common with ‘syndrome X’ such as hypertension, obesity and insulin resistance, they may also experience increased cardiovascular consequences of these features – termed “syndrome Z”–as described by Wilcox et al. in 1998.\textsuperscript{18}

In the last decade, several groups have described a relationship between the diagnosis of sleep disordered breathing (both OSA and CSA) and AF.\textsuperscript{19-21} The underlying mechanisms proposed to be contributory including repetitive sympathetic discharges, activation of neuro-hormonal and inflammatory pathways, cardiac structural changes, and the effects of associated disease such as hypertension. Together these are thought to result in potential triggers for the initiation of AF, and
via adverse atrial myocardial remodeling, perpetuation and long term maintenance of the arrhythmia.

This work, therefore, aims to characterise the clinical presentation of symptomatic sufferers of AF with particular reference to the impact of OSA, highlighting the acute and chronic effects of this disease on the atrial myocardium.

1.2 OVERVIEW OF THESIS

This introduction provides an overview of the current literature. The history and epidemiology of AF and OSA, as well as their pathophysiology and the mechanisms linking the two diseases will be discussed. This thesis does not address the relationship between AF and central sleep apnoea. Clearly this is another topic worthy of separate study.

In Chapter 2, a study will be presented delineating the differences in substrates between patients with paroxysmal and persistent AF. The aim of this chapter is to highlight some differences in the remodeling of the atria in AF. This project did not take into account new potential risk factors such as OSA, but is important as it demonstrates significant remodeling in the presence of AF maintenance.

In Chapter 3, a study of patients presenting for invasive management of AF with radiofrequency ablation will be presented. This study will document the incidence of OSA in this symptomatic population, and the impact of the sleeping disorder on the patients’ presentation, and, outcomes of invasive therapy.
Chapter 4 will further examine this population of patients and directly compare the electroanatomic features of patients with AF presenting for ablation, dichotomised on the basis of the presence of moderate-severe OSA. This study will, for the first time, present direct clinical evidence of more advanced atrial remodeling in the presence of OSA and AF.

Chapter 5 will present data on the effect of acute OSA episodes on atrial electrophysiology during an overnight stay in hospital after ablation of AF. This will highlight acute electrical changes that occur in the presence of untreated OSA and provide further evidence for the nocturnal finding of AF in this group of patients.

Finally, Chapter 6 will present an ex vivo rabbit model, highlighting the effect of hypoxia and hypercapnia on atrial electrophysiology and how this may contribute to the pathogenesis of AF in OSA.

Tables and figures for each chapter appear at the end of each chapter. A single bibliography appears at the end of the thesis.

1.3 ATRIAL FIBRILLATION

1.3.1 A HISTORICAL PERSPECTIVE

The earliest record of AF comes from the *Yellow Emperor’s Classic of Internal Medicine* in the 17th century (Huang Ti Nei Ching Su Wen). Huang Ti, the legendary emperor physician (said to be the third of China’s first five rulers) is believed to have ruled China between 2696 and 2598 BC. The *Nei Ching* is thought to be a reworking of an earlier version completed in 762 A.D but may have been in existence as far back as the Han dynasty (206 B.C–25 A.D). The first description of
“auricular fibrillation” in animals, however, comes from James Harvey in 1628, where he observed undulation of the blood in the right atrium of a dying dog, which continued long after the ventricles had stopped beating.\textsuperscript{24}

In the 19\textsuperscript{th} century, “Ataxia of the pulse” – heartbeats with unequal intervals and equal or unequal force, was discussed in a publication by Bouilland (1835).\textsuperscript{25} Shortly thereafter, the “kymograph” (which literally means ‘wave writer’), developed by the German physiologist Carl Ludwig in the 1840’s, alongside further observations from German physiologist Vierordt on blood pressure and pulse, allowed graphical representation of the an irregular pulse referred to as “delirium cordis”.\textsuperscript{26} Using sphygmography to record the pulse graphically, Chauveau and Marey in 1863 described via several experiments, the irregular pulse tracings of AF.\textsuperscript{27} Similarly, a ‘Jacques sphygmochronograph’ was used by Cushny and Edmunds to describe postoperative atrial fibrillation in 1907, the first case report of AF.\textsuperscript{28} Further physical findings were presented by James Mackenzie in his study on “affections of the heart”, describing the absence of the ‘auricular wave’ in conjunction with a continuously irregular rhythm.\textsuperscript{29}

The association of AF with another disease is historically best described in mitral valve disease. “Rebellious palpitations” associated with mitral stenosis was described by Jean Baptiste Seac in 1783,\textsuperscript{30} and further descriptions came from Irishman, Robert Adams, in 1827.\textsuperscript{31}

In 1902, Willem Einthoven, a Dutch doctor and physiologist, invented the first practical electrocardiogram using a fine quartz string coated in silver (the string galvanometer), for which he received the
Nobel Prize in Medicine in 1924. He recorded ‘pulsus inequalis and irregularis’ using 26 ECG strips with transmission of electrical signals over telephone wires to his laboratory. This was followed some years later with the classic description of ‘irregular waves clearly seen in diastole’ published in the 1909 work of Thomas Lewis. ‘Pulsus irregularis perpetuus’, a similar description to that proposed by Lewis, was simultaneously published by Rothberger and Winterberger in Vienna. In subsequent years came the miniaturisation of the ECG recording system, pioneered by Frank Sanborn’s company (later bought by Hewlett-Packard in 1961 and then by Philips Medical Systems in 1999) which set the scene for mainstream documentation of the disease by medical practitioners.

1.3.2 EPIDEMIOLOGY OF ATRIAL FIBRILLATION

Atrial fibrillation is the most common sustained arrhythmia, affecting 2.3 million Americans. Eugene Braunwald previously used the term ‘epidemic’ to describe the rising prevalence of AF in the population. According to recent US population projections, the number of people with AF will exceed 12 million by the year 2050 if the increase in incidence continues. The current lifetime risk for the development of AF is 1 in 4 for both men and women aged 40 years, and even in the absence of risk factors such as congestive heart failure or myocardial infarction, the overall lifetime risk for the development of AF is 1 in 6 for both men and women. The implications of this are staggering considering the significant morbidity attributed to AF, including thromboembolic stroke, congestive heart failure, cognitive dysfunction and an increased mortality rate.
Age is recognised as having a major effect on the prevalence of AF. In the general population, the prevalence of AF is 1%, however, prevalence increases from 0.1% among adults <55 years, to 9% among those over the age of 80,\textsuperscript{2,43,44} with approximately 70% of all individuals with AF aged between 65-85 years.\textsuperscript{46} By 2050, it is expected that 53% of patients with AF will be over the age of 80.\textsuperscript{2}

The Cardiovascular Health Study\textsuperscript{46} reported that the prevalence of AF in the age group 65-69 years was 5.8% in men and 2.8% in women. However, in the age group 70-79 years, the prevalence was almost equal in men and women (5.9% and 5.8% respectively).\textsuperscript{46} Whilst men have a 1.5 times higher risk of developing AF then women,\textsuperscript{47} the absolute number of men and women with AF is probably equal. This can be explained by the relatively greater longevity of women.\textsuperscript{45}

In 2004, it was estimated that the number of Australians with AF was 165,000.\textsuperscript{48} Furthermore, ‘atrial fibrillation and flutter’ listed as a principal diagnosis on discharge from public hospitals increased from 27,245 to 38,296 between 1999 and 2005.\textsuperscript{49} More recent data presented in a review of 510,424 hospitalisations by Wong et al., identified 23,125 principal and secondary AF diagnoses, and documented that 25.6% of patients discharged with AF were readmitted to hospital in the same year.\textsuperscript{4}

The significant morbidity resulting from AF includes stroke, heart failure and an increase in all-cause mortality (approximately double that seen in sinus rhythm.\textsuperscript{50,51}), counteracting the survival advantage for females.\textsuperscript{43,52,53} Stroke, the most feared association with AF, in increased 5-fold in the presence of the arrhythmia.\textsuperscript{39} In the US, it accounts for 45
deaths in every 100,000 people, and approximately 795,000 people experience a new or recurrent stroke per year—87% being ischaemic.\textsuperscript{54} Globally, stroke causes nearly 10% of all deaths and is responsible for more than 4% of direct health care expenditure.\textsuperscript{55,56} In Australia, AF also represents a major health problem, contributing to a substantial proportion of the estimated 50,000 strokes per year.\textsuperscript{57,58}

The Olmsted Country Study of 4,498 AF cases reported 3,504 admissions to hospital over a 3 year period with 10.5% attributable to thromboembolic events.\textsuperscript{59} From the same population, 11\% of the 4,117 followed patients sustained a stroke during a mean follow-up time of 5.0±5.0 years. This study also showed that, despite a reduction in stroke seen over the 20 year period of follow-up (due to the introduction of anticoagulation), there was no reduction in the relative mortality risk associated with stroke when compared to the general Minnesota white population (HR 1.88 for men; 1.84 for women) without stroke, and with stroke (HR 3.03 for men; 3.80 for women).\textsuperscript{60}

The Renfrew/Paisley study,\textsuperscript{52} conducted between 1972 and 1976 on 7,052 men and 8,354 women in the west of Scotland, found that over a 20 year period, there was a 5-fold increase in the risk of any cardiovascular event (resulting in hospitalisation or death) in women, compared to a 2-fold increase in men. These events were mainly due to heart failure and stroke. On multivariate adjusted assessment, AF was independently associated with increased cardiovascular events and all-cause mortality (1.5-fold for men and 2.2-fold for women), and cardiovascular mortality (1.8 fold for men, 2.8 fold for women), with the association between AF and cardiovascular events being stronger in
women (p<0.001 for the interaction). In comparison, ‘lone’ AF—defined in this study as the absence of concurrent signs and symptoms of cardiovascular disease, conferred a non-significant increased risk of a cardiovascular event or death.\textsuperscript{52} Furthermore, in the Framingham study, the risk of stroke was found to be higher in patients with coronary artery disease, hypertension and heart failure. In the presence of AF and one of these three comorbidities, the risk was doubled in men and trebled in women.\textsuperscript{39} This study also highlighted the importance of age in association with AF as a risk factor for stroke. With advanced age, AF becomes increasingly prominent as a risk factor—the attributable risk being 1.5% in the sixth decade and 23.5% by the ninth decade of life, when it is the only significant cardiovascular risk factor for stroke.\textsuperscript{39}

Heart failure is the most common complication of AF, with a prevalence of between 13-27% among AF patients.\textsuperscript{61-65} In one study examining new AF or heart failure in subjects from the Framingham study,\textsuperscript{41} the incidence of heart failure among AF sufferers was 33 per 1000 person-years, and the incidence of AF in heart failure was 54 per 1000 person-years. There was an increase in mortality in AF subjects with the development of heart failure (HR 2.7 for men; HR 3.1 for women), and similarly, in patients with pre-existing heart failure, the development of AF was associated with an increase in mortality (HR 1.6 for men; HR 2.7 for women).\textsuperscript{41}

After adjusting for age in the Framingham study, other independent risk factors of note for AF were diabetes (OR 1.4 for men, 1.6 fold for women), left ventricular hypertrophy (OR 1.4 for men, 1.3 for women), myocardial infarction (OR 1.4 for men; 1.2 for women), valvular
disease (OR 1.8 for men; 3.4 for women) and hypertension (OR 1.5 for men, 1.4 for women). In the Renfrew-Paisley study, the independent correlates with AF at baseline included cardiomegaly (OR 7.3 for men; 17.4 for women), chronic bronchitis (OR 2.2 overall), left ventricular hypertrophy (OR 4.2 overall), myocardial ischaemia (OR 2.2 for men; 5.6 for women), previous history of stroke (OR 3.9 overall) and blood sugar ≥ 7 mmol/L (OR 3.1 overall). In another large community study—the Cardiovascular Health Study—after following 5,888 subjects 65 years of age or older for more than 15 years, independent risk factors for AF in addition to old age included valvular heart disease (HR 2.42), increased left atrial size (HR 1.74 per cm), coronary artery disease (HR 1.48), use of diuretics (HR 1.51), increased systolic blood pressure (HR 1.11 per 10 mmHg), increased plasma glucose (HR 1.08 per mmol/L) and height (HR 1.15 per 5 cm). Whilst high levels of alcohol intake have been associated with an increased risk of AF, in the Cardiovascular Health Study, modest alcohol intake was protective against AF. Beta-blockers were also protective (HR 0.61).

The Framingham study also demonstrated that echocardiographic data from these patients can offer prognostic information for AF, in addition to the traditional risk factors—left atrial enlargement (39% increase in risk per 5mm increment in LA diameter), left ventricular fractional shortening (34% per 5% decrement), and left ventricular wall thickness (28% per 4mm increment). Moreover, for each echocardiographic predictor, risk increased progressively over successive quartiles. When these features were present in combination, the cumulative 8-year age-adjusted AF rates were 7.3% (one highest risk
quartile present), 17.0% (two high risk quartiles present) compared to 3.7% when none were present.\textsuperscript{69}

In the Framingham study, parental AF increased the risk of offspring developing the arrhythmia, suggesting a genetic susceptibility.\textsuperscript{70} This has been further supported by genetic studies identifying various culprit genetic defects, e.g. that linked to chromosome 10q in a Spanish family,\textsuperscript{71,72} and chromosome 6q14-16 in a 34 member family with Mendelian traits.\textsuperscript{73} Investigators have demonstrated that in the presence of a first degree relative with AF, the risk for an individual is significantly increased when compared to the general population.\textsuperscript{74}

Given the immense health burden associated with AF, there have been global attempts to understand its predisposing conditions, and develop more effective management strategies that might not only lower the patients’ symptomatic burden, but also reduce the associated morbidity and mortality.

1.3.3 PATHOPHYSIOLOGY OF ATRIAL FIBRILLATION

1.3.3.1 HISTORICAL OVERVIEW OF MECHANISMS

Understanding of the various mechanisms involved in the initiation and maintenance of AF has evolved over the last century. Three important (but competing) theories for the electrical basis of AF were highlighted in 1924 by Garrey.\textsuperscript{75} These were the ‘hyperectopia theory’, the single rotor or ‘mother wave’ theory, and the ‘multiple circuit reentry’ theory.

In 1907, Winterberg proposed that AF resulted from multiple firing foci distributed throughout the atria, after observing that individual
muscle fibres were capable of pulsatile activity.\textsuperscript{76} Further to this, Lewis and Schleiter proposed that rapid focal activity from one or more centres accounted for AF (multiple heterotopous centres theory).\textsuperscript{77} This was challenged by a series of observations made by Garrey in 1914, which contradicted the single rapidly firing focus concept. He also noted that a critical amount of cardiac mass was necessary to maintain fibrillating myocardium. In his observations of ventricular fibrillation, he observed that circus conduction is determined by the presence of 'blocks' and were the “essential phenomena of fibrillation”.\textsuperscript{78} Around the same time, Mines (using dogfish heart split up in such a way as to form a ring) advanced the circus movement theory of reentry, suggesting it to be an impulse circling a large anatomic obstacle. From this, he formulated some essential requirements of reentry– unidirectional block and the wavelength concept, whereby reentry is most likely to occur when conduction velocity is slow and refractory period duration is short.\textsuperscript{79}

Building on earlier observations by Lewis describing focal sources for AF,\textsuperscript{80} Moe and colleagues demonstrated that applying aconitine to the atrial appendage resulted in rapid tachycardia that could be halted when the appendage was clamped off. However, when AF was induced by rapid stimulation to the appendage and the atrial refractory period shortened by acetylcholine, whilst AF ceased in the clamped off atrial appendage, the rest of the atrium continued to fibrillate.\textsuperscript{81} From these observations, Moe formulated the 'multiple wavelet hypothesis'\textsuperscript{82} and, further to this, published his classical computer simulation study on AF using a two dimensional array of coupled excitable elements that had randomly distributed refractory periods.\textsuperscript{83} His hypothesis was that AF
could exist in a self-sustaining fashion independent of its initiating agency and was possible as a result of heterogeneously excitable tissue. This process required that a propagating impulse would render parts of the tissue partially or fully refractory, while other parts were excitable by the succeeding activation wavefront.\textsuperscript{83} As such, a train of external stimuli with extremely short coupling intervals of almost the same length as the minimal refractory period of myocardial cells resulted in some cells not recovering in time because their refractory period exceeded the stimulation period. Hence, fragmentation of the excitation waves ensued and multiple wavelets formed which could then wander randomly through the myocardium. Furthermore, this computer model demonstrated that, as the waves break up on encountering refractory cells, daughter wavelets are formed.

In an interesting extension of Moe's work, Krinsky et al. determined the necessary conditions for self-sustaining activity on the basis of 'critical mass of fibrillation' and 'extent of tissue heterogeneity'.\textsuperscript{84} To summarise, factors promoting AF in this model were shortening of refractory periods, increasing tissue refractoriness, heterogeneity and slowing of conduction, with the opposite tending to provide a substrate less capable of maintaining the multiple wavelets.

In 1977, in a study on the isolated rabbit left atrium, Allessie et al. provided further insight into the nature of reentry with work demonstrating areas of "block" around which circus movement could occur.\textsuperscript{85} In this experiment, rapid tachycardia could be induced by critically timed premature stimuli, and maintenance of the tachycardia was facilitated by adding carbamylcholine to the tissue bath, shortening
the refractory periods to about 40–50 ms. During the period of sustained tachycardia (around 30mins), spread of activation was observed across unipolar electrodes, allowing for accurate measurement of the excitation pattern during sustained tachycardia, as well as intracellular recordings from the centre of circus movement. From the results of this experiment, a new model of circus movement in cardiac tissue was born. Allessie proposed that where there is no anatomic obstacle to define the length of a circular pathway, it is "completely defined by the electrophysiological properties of the fibres composing the circuit". Hence, according to this, the "leading circle" is the smallest circuit possible in which the "impulse can continue to circulate in a circuit in which the stimulating efficacy of the circulating wavefront is just enough to excite the tissue ahead which is still in its relative refractory phase". According to Allessie, the length of the circular pathway is equal to the wavelength of the circulating impulse (the product of conduction velocity and refractory period). The area within the leading circle is activated by centripetal wavelets arising from the leading source of the circle and colliding in the very centre of the circus movement. In summary, electrophysiological properties of the tissue, such as conduction velocity and time course to recover excitability, all determine the length of the circuit.

Clinically, the multiple wavelet hypothesis and its critical requirements was exploited by the development of the Maze procedure, where permanent AF was managed by compartmentalisation of the atria into regions unable to support fibrillatory waves. Cox et al. published their five-year experience with this procedure in 1993, reporting that it
cured AF, restored atrioventricular synchrony and preserved atrial transport function in 98% of cases and was curative without the need for medications in 89%.86,87

Despite dominance of the multiple wavelet hypothesis, several investigators have presented evidence suggesting alternative contributors to the pathophysiology of AF. In their early experiment on the initiation of AF, Scherf et al. identified focal sources from the atrial appendage using aconitine, suggesting a role in the maintenance of AF.88 Premature systoles, paroxysmal tachycardia, flutter and fibrillation were investigated by Prinzmetal et al. in experiments on over 200 dogs using high-speed cinematography, cathode ray oscillography and multiple-channel electrocardiography.89 They concluded that a single ectopic focus was responsible for all arrhythmias examined. Waves did not pursue a circus path and no daughter waves were seen on the films.89 Wu et al. performed simultaneous biatrial computerised mapping during permanent AF on 6 patients with organic heart disease undergoing surgery.90 Their aim was to test the hypothesis that repetitive high-frequency activation sources were present in the LA but not in the RA, and hence, that an activation rate gradient exists between the two chambers. Indeed, they found that the LA posterior wall activation was characterised by rapid repetitive activity from the edge of the mapping plaque and that an activation rate gradient from LA to RA did exist during AF.90 Similar work by Skanes et al. in Langendorff-perfused sheep hearts demonstrated temporal and spatial stability in periodic waves, with the cycle-length of the LA sources determining the dominant peak in the frequency spectra.91 Mandapati et al. also showed spatiotemporal
periodicity during induced AF, with the highest dominant frequency most often localised to the posterior left atrium close to the pulmonary vein ostia, suggesting LA foci were important micro-reentrant sources.\textsuperscript{92}

Recently, Haissaguerre et al. examined patients with AF after prior ablation. It was found that 17\% had organised discrete electrograms, with a consistent activation sequence and localised to an area with centrifugal propagation to surrounding atrial tissue.\textsuperscript{93} Their importance in the maintenance of AF was demonstrated by prolongation of the cycle length and/or termination of AF on radiofrequency ablation of the focus.

In a canine model of rapid atrial pacing, Sih et al. noted differences in the frequency of AF, with faster frequencies present in persistent AF and slower frequencies in paroxysmal AF.\textsuperscript{94} Lazar et al. further demonstrated a left-to-right atrial frequency gradient and, interestingly, the gradient was not demonstrable in the presence of more persistent AF, suggesting a more important role for the posterior LA in paroxysmal but not persistent disease.\textsuperscript{95} Sanders et al. highlighted the different distributions of dominant frequencies in 32 patients undergoing radiofrequency ablation of AF. In this study, paroxysmal AF was associated with dominant frequencies in the pulmonary veins, whereas atrial sites with dominant frequency were found in patients with permanent AF. When targeted with ablation, termination was achievable in 15 out of 19 patients with paroxysmal AF, and, 69\% of these patients were rendered non-inducible for the arrhythmia.\textsuperscript{96}
1.3.3.2 IMPORTANCE OF THE PULMONARY VEINS

Focal atrial tachycardia was been well described in the literature. It is thought to arise from cardiac cells exhibiting automaticity, triggered activity or reentry.\textsuperscript{97} These tachycardias tend to cluster close to anatomical structures such as the crista terminalis,\textsuperscript{98} tricuspid annulus,\textsuperscript{99} ostium of the coronary sinus,\textsuperscript{100} right atrial appendage,\textsuperscript{101,102} mitral annulus,\textsuperscript{103} left atrial appendage,\textsuperscript{104,105} left atrial septum,\textsuperscript{106} and the ostia of the pulmonary veins.\textsuperscript{107}

The most important observations with regard to ectopic foci were those made by Haissaguerre et al. in the seminal study from 1998 on the critical role of the pulmonary veins.\textsuperscript{5} In this study on 45 patients with frequent episodes of AF refractory to drug therapy, a single point of ectopy was identified in 29 patients with the other 16 having either 2 or 3 points identified. The majority of these (94\%) were in the pulmonary veins with a predilection for the superior veins. Local depolarisations preceding the atrial ectopics were found within the pulmonary veins and AF was initiated by a sudden burst of rapid depolarisations. The pulmonary vein foci were unpredictable and demonstrated complex delayed conduction to the LA. Their origin was from within the pulmonary veins as opposed to the juxtaostial origin commonly seen in atrial tachycardias. Radiofrequency ablation of the pulmonary vein foci was successful in eradicating AF in 84\% of cases during hospital monitoring and 62\% on discharge, demonstrating the importance of these ectopics in the aetiology of the AF.

Histological studies have shown sleeves of myocardial bundles extending into the pulmonary veins in complex spatial orientations (e.g.
longitudinal, circular, oblique and spiral), heterogeneously interspaced with nonconducting connective tissue over variable distances, the longest being in the superior veins. The variation in myocardial fascicle orientation interspersed with the nonconduction tissue may facilitate reentry on the bases of conduction delay and slowing. This was demonstrated in a study by Hocini et al. on Langendorff-perfused dog hearts, where activation delays in conduction after premature stimulation were found. Other have shown that the myocardial architecture of the pulmonary veins and the pulmonary vein-atrial junction allows for overlapping of the muscle fibres, with the smooth venous walls in a gradual transition with islands of fibrous tissue forming gaps, creating an environment capable of sustaining reentry. Whilst a substrate for reentry is present, other potential mechanisms are suggested by recent studies on the properties of the myocardial cells within the pulmonary veins of rabbits. These have shown some features in common with cells from their sinoatrial nodes. Interestingly, the cells did show spontaneous activity as well as activity in response to stimulation at physiological rates. Histological and immunostaining preparation from human heart autopsy specimens have identified ‘Cajal cells’ in 3 out of the 8 specimens, 2 of which had a history of AF, and one of the AF patients demonstrated the highest density in a pulmonary vein. Others have shown that pale (P) and Purkinje cells are observed in the pulmonary veins of rats, dogs, and humans. Interestingly, work by Blom et al. on the development of cardiac conduction tissue showed transient staining using HNK-1 immunohistochemistry (which stains the developing atrioventricular conduction system) of myocardium
around the pulmonary veins, suggesting embryonic origins of pacemaker
cells in the pulmonary veins.\textsuperscript{120}

To summarise, a combination of reentrant and non-reentrant
mechanisms (triggered and automatic) are likely to be present in the
pulmonary veins, and eradication of their arrhythmogenic potential
provides the rationale for the current approach to catheter ablation of AF
with or without other atrial modification strategies.\textsuperscript{121}

1.3.3.3 TRIGGERS, SUBSTRATE AND MODULATORS

Human AF results from a complex interaction between triggers,
functional and structural myocardial substrates, and modulators of these
components. In paroxysmal AF, the role of the pulmonary veins has
been better described since the landmark observations of Haissaguerre.\textsuperscript{5}
Indeed, there is evidence to suggest that these structures have a role not
only in the initiation but also the substrate for paroxysmal AF. However,
in persistent AF, while they are recognized to be one source of triggers
initiating AF, their contribution as a whole to the sources of triggers and
the substrate is not well defined. With the substantial body of knowledge
developed over recent years and greater acceptance of ablative
technologies, has come the realisation that extensive lesion sets are
needed beyond isolation of the pulmonary veins, especially when more
persistent arrhythmia is present. The maintenance of AF appears to rely
more heavily on an underlying myocardial substrate as the disease
progresses from its paroxysmal to its persistent form.\textsuperscript{122-124} Several
studies have observed patients with paroxysmal AF at baseline progress
to permanent AF over time. In one study on a population from Olmstead
County, there was a 30-year cumulative probability of 29\% (95\% CI 16-
42), the progression in most of these patients occurring in the 15 years after diagnosis. These studies suggest the development of a substrate over time, i.e. atrial remodeling.

1.3.3.3.1 NORMAL ATRIAL ELECTROPHYSIOLOGY

Five phases of a normal action potential are present in the human myocyte: phase 0 - rapid depolarisation; phase 1 - rapid repolarisation; phase 2 - the plateau phase; phase 3 - phase of rapid repolarisation that restores membrane potential to its resting value; and phase 4 - being the resting potential stable at ~90mV.\textsuperscript{125} Differences exist in the electrophysiological basis of the cellular action potential between ventricular and atrial myocytes, mainly based on the current density and kinetics of the repolarising currents. Atrial action potentials exhibit more of a triangular shape with a gradual repolarisation phase, and a less pronounced plateau phase than seen in ventricular myocytes.\textsuperscript{126} Action potentials show some regional variation within the atria, with the longest action potential duration (APD) seen in the crista terminalis and shorter action potentials seen closer to the atroventricular groove. The differences are manifested by the relative sizes of the different ionic currents.\textsuperscript{127,128} Some areas also demonstrate endocardial-epicardial heterogeneity in action potential duration such as around the crista terminalis, which exhibits shorter epicardial APDs secondary to higher transient outward potassium current (\(I_{to}\)) density.\textsuperscript{127,128} Specialised conduction tissue exists in the atria, such as Bachmann’s bundle, where cells exhibit higher conduction velocity, longer action potential plateaus and longer refractory periods.\textsuperscript{129,130}
In atrial myocytes, the currents responsible for depolarisation are the rapidly activating/inactivating sodium (Na+) current \((I_{Na})\) and the L-type calcium (Ca2+) current \((I_{Ca})\), which accounts for both the shorter plateau and the triggering of intracellular Ca2+ stores responsible for the contraction of the cell. The resting membrane potential is set by the inwardly rectifying potassium current \((I_{K1})\). A number of potassium channels contribute to both early \((I_{to}, I_{kur})\) and late \((I_{Ks}, I_{Kr}, I_{Kur}, I_{K1})\) repolarisation.

Depolarisation is carried from cell to cell connections via gap junction proteins called connexins (Cx)–namely Cx40 and Cx43, of which Cx40 is exclusively present in the atria. These two connexin subtypes have been shown to combine forming heteromeric channels.

1.3.3.3.2 ANIMAL STUDIES ON ATRIAL REMODELING

1.3.3.3.2.1 ATRIAL FIBRILLATION

The evolution of our knowledge of AF mechanisms has identified two fundamental components underpinning AF – tissue refractoriness and conduction time. For reentry to be maintained, conduction time must be greater than the longest refractory period of the circuit, to allow the impulse to traverse the entire circuit slowly enough for all the cells to regain excitability. Hence, susceptibility is governed by longer path lengths, slower conduction velocity and shortening of refractoriness.

In a canine model of AF, Morillo et al. measured baseline effective refractory period (ERP) and vulnerability to AF via programmed atrial electrical stimulation at baseline in 22 dogs. After other instrumentation was removed, the right atrial (RA) appendage was paced at 400bpm for 6 weeks via a retained wire. Chronic atrial pacing
resulted in remodeling to the atria characterised by biatrial enlargement, accompanied by increases in P-wave duration, reduction in the endocardial RA ERP, progressive shortening of the AF wavelength, maladaptation of the atrial ERP, and sustained AF with programmed electrical stimulation.\textsuperscript{137}

Wijffels et al., in a conscious goat model, used 1 second bursts of biphasic stimuli to maintain AF continuously.\textsuperscript{123} Where goats had been in sinus rhythm, electrically induced AF lasted only a short period. However, where AF was maintained for longer periods, AF persisted for much longer. After two weeks, AF became sustained and ceased to terminate spontaneously, leading to the phrase “AF begets AF”. By comparison, it was noted that conduction velocity measured over Bachmann’s bundle, did not change during the experiment.

Again, atrial ERP reduction and a loss of accommodation to rate were demonstrated by Gaspo et al. in a model where dogs were paced for either 1, 7 or 42 days.\textsuperscript{138} The changes predominantly occurred within the first seven days. Here, atrial conduction velocity did change, however the slowing occurred more gradually compared to the ERP change and was maximal by the 42\textsuperscript{nd} day. Stepwise linear regression highlighted the importance of both wavelength and duration of pacing in determining the AF duration.

Morton et al. documented similar time-delay in the slowing of conduction during studies on atrial flutter in an ovine model, where ERP’s declined by day 3. However a gradual and slower reduction in conduction velocity was recorded over the full 28 day experimental period.\textsuperscript{139}
Other investigators have shown that even very short episodes of AF result in electrical remodeling, such as in the study by Daoud et al.\(^{140}\) Here, with induced AF durations of only 7.3 minutes, a reduction in the ERP was noted for up to 8 minutes, and, as ERP recovered, only shorter episodes of AF were inducible. Sparks et al. demonstrated similar reduction and recovery of ERP over 5 minutes after short bursts of arrhythmia in patients with paroxysmal atrial flutter.\(^{141}\)

The chronic effects of AF have been demonstrated by Kumagai et al.\(^{142}\) In this study, it was noted that on electrical cardioversion of patients with chronic lone AF without atrial structural changes such as chamber dilatation, when compared to those without AF, patients demonstrated decreased ERP’s, sinus node dysfunction, longer P-wave duration (as a surrogate for total atrial activation time) as well as conduction delay and fragmented atrial activity. This suggested an altered underlying myocardial substrate was present, promoting chronicity of the arrhythmia.

Other studies have demonstrated that dispersion of the ERP is important (i.e. nonuniformity of local atrial refractory periods resulting in juxtaposition of regions with short ERP to areas with much longer ERP without the necessity of gradual transition). This was observed by Nattel’s group (in dogs subjected to rapid atrial pacing), which found ERP heterogeneity was an independent determinant of inducibility and duration, whereas the actual ERP was not.\(^{143}\) In these models, mapping demonstrated local conduction slowing and/or block in zones with longer ERP adjacent to areas of shorter ERP where AF was inducible. Variability of ERP was seen both among, and, within different atrial
regions, contributing to the heterogeneous nature of the AF-induced remodeling, leading to perpetuation of the arrhythmia.

In examining the importance of dispersion of refractoriness on the inducibility and maintenance of AF in 47 patients, Oliveira et al. found that increased dispersion was a marker of vulnerability to arrhythmia in AF, but that the degree of dispersion was not related to the duration of the sustained arrhythmia.\textsuperscript{144,145} This suggested that perhaps other factors were important to the maintenance of AF.

Reversal of these features secondary to electrical remodeling has also been extensively examined. As noticed by Wijffels et al. in their chronically instrumented goat model, all electrophysiological alterations induced were returned to normal baseline levels within 1 week of restoration to sinus rhythm.\textsuperscript{123} Garratt et al. also noted that goats with AF maintained for 5 days had atrial refractoriness reversed to baseline levels without any residual increased inducibility of AF after restoration of sinus rhythm for 48 hours.\textsuperscript{146} Hobbs et al. examined AF cycle length before and after internal cardioversion in patients with persistent AF.\textsuperscript{147} They documented a reversal of the altered atrial electrophysiology with a post-cardioversion increase in AF cycle length positively correlating the duration of sinus rhythm before recurrence of AF.

Recovery within the atrium does not appear to be uniform however, as was demonstrated by Lee et al. who assessed electrophysiological properties at 7 epicardial sites over the RA and left atrium (LA).\textsuperscript{148} They found that during 48 hours of measurements, there was progressive recovery of the RA ERP and return of rate adaption, with a decrease in the duration and inducibility of episodes of AF. These
changes were slower to occur in the LA compared to the RA and Bachmann’s bundle.

Atrial refractoriness is dependent on the cardiac APD and this in turn is determined by the balance between inward Ca\textsuperscript{2+} currents keeping the cell depolarised and outward K\textsuperscript{+} currents tending to repolarise.\textsuperscript{131} As described above, tachycardia itself may alter atrial electrophysiology by causing several changes to the ions regulating depolarisation and repolarisation.\textsuperscript{149} This was described by Yue et al. on action potential changes in the atria of dogs during short and long term tachy-pacing. They documented changes in outward $I_{to}$ and L-type calcium ($I_{cal}$) currents with the continuation of pacing, resulting in reduction of the APD and shortening of refractoriness, suggesting a protective mechanism counteracting cellular loading.\textsuperscript{150-154}

AF is also associated with spontaneous Ca\textsuperscript{2+} release from the sarcoplasmic reticulum–Ca\textsuperscript{2+} “sparks and waves” resulting from upregulation of the sarcoplasmic reticulum Ca\textsuperscript{2+} release channel activity.\textsuperscript{155} Ca\textsuperscript{2+} handling in atrial tachy-pacing models as well as AF patients has also been implicated in the loss of rate-adaptation to APD.\textsuperscript{156} Potassium current (K\textsuperscript{+}), also remodelled by atrial tachycardia, has an additional effect on APD and thus refractoriness. $I_{to}$ is down-regulated by persistent atrial tachycardia, however, its functional importance in this regard is not clear as only small changes in human APD have been demonstrated.\textsuperscript{157,158} The ultrarapid delayed rectifier potassium current ($I_{kur}$), an atrial specific current in humans, and thus thought to be important, has largely shown inconsistent changes during AF. However, as the APD shortens, the contribution of $I_{kur}$ to
repolarisation increases despite its downregulation.\textsuperscript{157-160} Delayed rectifier K\textsuperscript{+} channel subunits have also been shown to be affected in AF patients, although their importance is not well documented.\textsuperscript{161} Several studies have now demonstrated a more important role for up-regulation of the inward rectifier K\textsuperscript{+} channel ($I_{K1}$) and the acetylcholine dependent K\textsuperscript{+} channel ($I_{KACch}$), as well as their role in APD shortening in promotion of AF.\textsuperscript{152,157,158,162-165} Furthermore, ischaemia-induced changes in the ATP sensitive K\textsuperscript{+} current also play a role in ischaemia mediated atrial electrophysiology changes, as demonstrated in simulated experiments and with channel antagonists.\textsuperscript{166,167} Transcriptional down-regulation at an mRNA and protein level has also been demonstrated for voltage-dependent K\textsuperscript{+} channel subunits.\textsuperscript{168-170} Sodium currents ($I_{Na}$) have been given less attention in the literature. However the upstroke of the action potential, governed by $I_{Na}$ is reduced with atrial tachy-pacing with resultant slowing of conduction, shortening of wavelengths and thus susceptibility to reentry.\textsuperscript{168,170}

Cx40 and Cx43 have been studied extensively, however, results are inconsistent. For Cx40, some studies demonstrate increased expression on the lateral surfaces of cells and increased heterogeneity.\textsuperscript{171-173} Other studies have demonstrated decreased Cx40,\textsuperscript{174,175} whilst some have shown no change in Cx40.\textsuperscript{174,176} Similar controversy exists for Cx43, which is more important in ventricular arrhythmias.\textsuperscript{177} Cx40 knock-out mice studies do suggest that Cx40 is a dominant connexin affecting atrial electrophysiology.\textsuperscript{178} Here, Cx40-/-mice showed prolonged P wave duration on electrocardiography, and atrial arrhythmias. Remodeling of gap junctions, with heterogeneity in
connexin expression probably results in heterogeneous intercellular coupling, leading to non-uniform conduction and anisotropy promoting reentry and thus, AF.\textsuperscript{171-173,177}

Pulmonary and non-pulmonary vein ectopy have been shown to be instrumental in the initiation and maintenance of AF.\textsuperscript{5,179,180} With regard to the pulmonary veins, studies have demonstrated specific electrophysiological properties of the action potential profiles and ionic current properties, allowing for abnormal Ca2+ handling with resultant early and delayed after-depolarisations. These are perhaps responsible for their high arrhythmogenic activity.\textsuperscript{181,182} Indeed, early animal studies demonstrated node-like cells with pacemaker like activity as a source of pulmonary vein ectopy.\textsuperscript{117,183} Remodeling of the pulmonary veins may also lead to promotion of ectopic activity as is seen in heart failure and sustained tachycardia models.\textsuperscript{184,185} AF itself has been shown to remodel the pulmonary veins characterised by lower signal voltages, slower conduction velocities and lower ERPs, moreover, this was more notable in patients with persistent arrhythmia compared to paroxysmal AF.\textsuperscript{186}

To summarise, electrical remodeling, as demonstrated through studies examining the effect of AF on the atrial myocardium, may promote the arrhythmia by shortening of the refractory period and slowing of conduction, mediated by a complex interplay between Ca2+ handling, K+ currents, Na+ currents and connexin expression. This disturbs the electrical coupling of myocytes and their cable like conduction properties. Enhanced remodeling may lead to alterations in cellular function, which may make cells such as those in the pulmonary
vein ostia prone to ectopy, and AF, once initiated, may be propagated by an environment now capable of reentry.

1.3.3.3.2.2 HEART FAILURE

In felines with spontaneously occurring cardiomyopathy, those animals with severely dilated left atria were noted to have the greatest number of inexcitable cells on microelectrode array studies. Histological specimens revealed diseased atria characterised by marked structural abnormalities such as interstitial fibrosis, cellular hypertrophy and thickened basement membranes. Congestive cardiac failure induced in dogs via ventricular pacing at rapid rates has also demonstrated substantial heterogeneity in velocity due to areas of discrete slowing of conduction associated, again, with histological evidence of interstitial fibrosis. On an electrophysiological level, changes were later documented as a decrease in density of $I_{CaL}$ by 30% and $I_{to}$ by ~50%, with the action potential of atrial myocytes increased at faster pacing rates, all of which contributed to an increase in documented AF duration.

Although, in the study by Li et al, there was no significant change in the ERP, changes in ERP have been documented with CHF by others. In an ovine model of cardiomyopathy induced by anthracycline exposure, LA dilatation associated with longer P-wave duration was found. Electrophysiological changes included a higher ERP irrespective of atrial location studied, and slowing of conduction associated with increased conduction heterogeneity. Histological preparations showed an increase in atrial fibrosis, and together, these changes were associated with an increase in the duration of AF,
indicating a marked effect of the atrial substrate secondary to the cardiomyopathy.

1.3.3.3.2.3 AGEING

Microelectrode techniques have been used in a canine model of ageing, comparing dogs aged >8 years to those aged <5 years. Conduction velocity of drive train beats was not found to differ between the 2 groups, however, early premature impulses demonstrated a slowing of conduction, and this was associated with a doubling of detected fibrous tissue. Changes were also noted with prolongation of the APD and spatial heterogeneity of repolarisation.

1.3.3.3.2.4 HYPERTENSION

Open chest evaluation of an ovine model of hypertension induced by corticosteroid exposure has demonstrated significant electroanatomic changes, contributing to an increased propensity to AF. These included increased conduction heterogeneity without changes in refractoriness. Associated with these electrophysiological findings was demonstration of atrial myocyte hypertrophy, and myolysis and apoptosis, with tissue fibrosis noted in 50% of the hypertension sheep. More recent ovine studies by Lau et al. have further characterised the widespread effect of hypertension on the atrial myocardium. In one model, 10 sheep had induced hypertension for 7±4 weeks after one kidney was surgically clipped. A 128-electrode epicardial plaque was used to examine the electrophysiological parameters of the atria and cardiac MRI was used to assess functional changes. Study sheep atria were enlarged and exhibited reduced mechanical function on MRI. Electrically, they were characterised by higher mean ERPs, slower
conduction velocity and higher conduction heterogeneity. Histological analysis showed cell infiltrates consistent with inflammation and an increase in interstitial fibrosis.

In a similar study on chronically induced hypertension by a similar method, this time examining 21 study sheep and 11 controls via a closed chest model, Lau et al. demonstrated progressive changes in the atria over time.\textsuperscript{197} Here, hypertension led to greater atrial dilatation and a reduction in atrial function, with greater inducibility of AF noted early. Electrophysiology studies demonstrated an increase in the conduction heterogeneity associated with conduction slowing, and histology specimens stained positive for fibrosis after 10 weeks. These later changes were associated with longer duration AF. There was correlation of the conduction abnormalities with AF inducibility, duration, and tissue inflammation/fibrosis. Together, these studies elegantly demonstrate a relationship between hypertension induced substrate change, altered atrial electrophysiology, and increased AF inducibility and duration.

1.3.3.3.2.5 HYPOXIA, HYPERCAPNIA AND OSA

Lammers et al. first performed an analysis of rabbit atria by microelectrode array, with freshly excised tissue placed in a physiological tissue bath.\textsuperscript{198} A microelectrode array was used to demonstrate the effect of pacing at different rates and from different sites over the left atrium. With atrial extrastimuli, phase mapping demonstrated a decrease in conduction homogeneity and this correlated with the presence of inducible atrial arrhythmia. In a study based on the same model, hypoxia was introduced with changing composition of the physiological bath by gassing the solution with a mixture of 95% N\textsubscript{2}.\textsuperscript{199}
This resulted in a marked increase in the frequency of induction of arrhythmias over a 60-minute period. Analysis showed an initial increase in the refractory period over 20 minutes followed by a decrease to 72% of the original ERP at 30 minutes of hypoxia. There was also a marked heterogeneity in refractoriness noted between sites and this correlated with the ability to induce reentry. Conduction velocity was similarly changed in the first 10 minutes with a reduction compared to the control value. Conduction heterogeneity was significantly increased in the hypoxic atria compared to the controls and arcs of conduction blocks were shown to account for changes in activation sequence.

An ovine model of sleep apnoea investigating the effects of hypoxia and hypercapnia, used a 64-electrode endocardial basket catheter positioned in the RA and 2x128 electrode epicardial plaques over the right and left atrial appendages, to record atrial activity during induced hypoxia and hypercapnia after autonomic blockade.\textsuperscript{200} Hypercapnia was associated with a lengthening of baseline ERP and an increase in the conduction time, which unlike refractoriness, demonstrated delayed recovery. AF inducibility was initially reduced due to the prolongation of refractoriness during the hypercapnic insult. However, AF inducibility increased significantly with a return of ERP to baseline in the presence of ongoing conduction slowing. Interestingly, this study failed to identify any significant changes in ERP, conduction time or inducibility of AF with hypoxaemia.

In a recent study that aimed to shed light on the effects of OSA in particular, 17 pigs were intubated with negative tracheal pressure applied intermittently to simulate OSA.\textsuperscript{201} When negative tracheal
pressure was applied for 2 minutes, RA ERP was measured and monophasic action potentials recorded. All obstructive events resulted in a reduction in the ERP and monophasic action potential. With resolution of obstruction, there was rapid return the ERP to baseline levels. During application of the pressure there was also enhanced AF inducibility with single premature beats. The changes were prevented by the use of atropine or vagotomy, suggesting that the mechanisms were clearly vagal mediated.\textsuperscript{202} As an extension to this study, the same authors investigated the cellular mechanisms involved in the above pathophysiology and found that only combined blockade of the $I_{kr}$ and $I_{kur}/I_{to}$ channels (using sotalol and AVE0118) resulted in attenuation of the negative tracheal pressure induced ERP shortening and AF inducibility. Neither drug alone produced the desired endpoint, in keeping with previous studies demonstrating the inability of $I_{Kur}$ alone to terminate cholinergic AF.\textsuperscript{203}

1.3.3.3.2.6 CORONARY ARTERY DISEASE

In a model, examining atrial ischaemia (rather than hypoxia per se), occlusion of an atrial arterial branch was performed.\textsuperscript{204} This lead to changes in AF induction after burst pacing, from 57 to 803 seconds after half an hour, and 887 seconds after 3 hours. None of the control dogs had inducible AF. However, in the dogs with ischaemic atria, there were significantly longer episodes with longer duration of the insult. Atrial refractoriness was initially not affected. However, after 5 hours of ischaemia, atrial refractoriness became prolonged. Phase mapping examined conduction and demonstrated severe slowing in the ischaemic
zone. There was histological correlation with extensive areas of necrosis in association with these identified areas of conduction slowing.

1.3.3.3.3 HUMAN STUDIES ON ATRIAL REMODELING

1.3.3.3.3.1 AGE

The relationship of AF to age has been well documented in several studies, occurring in 44% of the population over the age of 65.\textsuperscript{45} Mapping studies performed by Kistler et al. on three groups of patients (≤30, 30-60, ≥60 years old) demonstrated significant changes in atrial electrophysiology that may account for this increased prevalence seen in population studies.\textsuperscript{205} With age, there was an increase in atrial ERP, however, refractory period dispersion was not seen to change with age. Other changes included prolonged conduction times seen with elongation of the surface P-wave, prolonged conduction along linearly placed catheters, as well as prolonged corrected sinus node recovery times (CSNRT). Conduction slowing was further seen on the creation of three-dimensional maps, which also demonstrated a reduction in endocardial voltage, as well as a greater number of double potentials and fractionated electrograms seen along the crista terminalis.

Changes in wavefront propagation velocities were further demonstrated by Kojodjojo et al. as having an inverse correlation to age.\textsuperscript{206} Complex fractionated electrograms were found in greater number among aged human atria, and were associated with other evidence of remodeling, including low atrial voltages and slowed atrial conduction. Fractionated electrograms recorded in sinus rhythm have also been correlated with advancing age, lower atrial voltages and a reduction in conduction velocity.\textsuperscript{207}
1.3.3.3.3.2 HYPERTENSION

Despite the relationship of hypertension to AF and the incidence of stroke, there are few studies demonstrating atrial remodeling in humans. \(^{208}\) Recently, Medi et al. published a study of 20 patients with hypertension and left ventricular hypertrophy, without a history of AF. \(^{209}\) They underwent electrophysiological evaluation of the RA. Compared to patients without hypertension/left ventricular hypertrophy, these patients had global conduction slowing with evidence of regional conduction delay at the crista terminalis. There was also evidence of myocardial thinning with a reduction in atrial voltages recorded. Moreover, despite the absence of AF, sustained AF was inducible in 30% of the hypertension patients compared to none of the controls.

1.3.3.3.3.3 CONGESTIVE CARDIAC FAILURE

Sanders et al. demonstrated the electroanatomic abnormalities present in the atria of patients with congestive cardiac failure. \(^{6}\) Patients with a low ejection fraction were compared to control subjects who had normal left ventricles and were presenting for ablation of supraventricular tachycardia. Electrophysiological measurements were taken from multiple sites including ERP, conduction time and CSNRT. Furthermore, a subset underwent electroanatomic mapping to determine atrial endocardial voltage, atrial activation, regional conduction velocity as well as fractionated electrograms, representing an abnormal underlying substrate. A potential substrate for AF was described, characterised by a homogeneous increase in ERP, an increase in the atrial conduction time measured along linearly placed catheters, on electroanatomic mapping, manifest on the surface ECG P-wave. Sinus node dysfunction was also
present. The substrate demonstrated low tissue voltages and areas of
electrical silence, which may represent underlying myocardial thinning
and scarring. There were also more electrograms that showed
fractionation and double potentials, suggesting areas of local conduction
block, again, a manifestation of the underlying substrate for AF.

1.3.3.3.4 SINUS NODE DISEASE

Luck et al. investigated atrial electrophysiology in 17 patients with
sinus node disease, with measurement of refractory periods at 3 sites in
the right atrium. The atrial myocardium demonstrated dispersion of
refractoriness. Interestingly 6 of their patients also had AF, but their
dispersion of refractoriness was not determined to be greater than those
without AF.

Patients with sinus node disease were more extensively
investigated by Sanders et al. to determine if an underlying substrate for
AF was present. Electroanatomic mapping was performed on 16
patients with symptomatic sinus node disease, comparing them to 16
age-matched controls. Patients with sinus node disease were found to
have altered electrophysiology compared to the reference patients,
manifest by higher ERPs and slower conduction times as well as more
localised areas of atrial electrogram fractionation. It was also found that
the sinus node complex itself had remodelled, and was more unicentric
and caudally shifted in the right atrium, along the low crista terminalis.
There were also low voltages and slowed regional conduction associated
with more extensive fractionated electrograms than in the reference
patients.
More recently Stiles et al. furthered the notion of a remodelled sinus node complex, by studying patients with supraventricular tachycardia and comparing them to patients with chronic atrial flutter.\textsuperscript{211} Using high-density simultaneous mapping techniques, they determined that conduction times via preferential pathways of conduction from the sinus node to the atrial myocardium were prolonged in the remodelled atrial myocardium of atrial flutter. The sinus node complex became more restricted, determined by shorter distances from the SVC-RA junction to the earliest activation of sinus break-out.

1.3.3.3.3.5 MITRAL VALVE DISEASE

Mitral stenosis resulting from chronic rheumatic heart disease is common in the developing world and is recognised as producing a significant predisposition to AF, with over 40% of affected individuals developing the arrhythmia.\textsuperscript{212} John et al. examined the electrical and structural abnormalities associated with mitral stenosis in a study of 24 patients with severe mitral stenosis, undergoing percutaneous balloon mitral commissurotomy.\textsuperscript{9} Again, multiple catheters were used to determine the electrophysiological properties of the diseased atria compared to a reference group, along with three-dimensional electroanatomic mapping. In summary, the abnormalities seen were in keeping with those described in other disease states, including left atrial dilatation with low voltages, electrical silence, and widespread fractionated electrograms. There was prolonged conduction and regional slowing, and site specific delay of conduction, with widespread double potentials along the crista terminalis. The ERPs in the diseased atria were either prolonged or not affected when compared to the reference
group. John et al. also later described the effects of chronic atrial stretch reversal by examining atria before and after mitral commissurotomy.\textsuperscript{213} Here, an immediate reduction in left atrial size and pressure was associated with an increase in the mitral valve orificial area. There were associated increases in conduction velocity and shortening of the P-wave duration on the surface ECG. Moreover, there was an increase in the atrial voltages measured on electroanatomic mapping. These variables continued to demonstrate positive change in the subset of patients that consented to invasive examination at a 6 month follow-up. These important data demonstrate that there are changes in the atrial myocardial, electrical and anatomical characteristics, with reduction in stretch (caused, in this case by mitral stenosis). This suggests a role for abnormal substrate reversal and thus a reduction in AF burden in treated patients.

Fan et al. also examined effects of percutaneous balloon mitral commissurotomy in patients with mitral stenosis and AF compared to mitral stenosis and sinus rhythm. They found that patients with AF had a different atrial substrate characterised by more prolonged and heterogeneous recovery of ERP and irreversible regional conduction delay after commissurotomy.\textsuperscript{214}

Roberts-Thomson also investigated the atrial abnormalities seen with chronic stretch, this time due to mitral valvular regurgitation.\textsuperscript{215} In contrast to the approach of Johns et al, this group examined patients at the time of open surgical intervention. Patients with mitral regurgitation with and without AF were selected for comparison against a reference group with normal left ventricular function undergoing coronary artery
bypass grafting. Epicardial mapping plaques were prepared and applied to the posterior left atrium. Conduction delay and conduction heterogeneity were more prevalent in the patients with mitral valvular disease and AF, as were fractionated electrograms in association with these areas of slow conduction velocity.

1.3.3.3.6 ATRIAL SEPTAL DEFECT

Atrial septal defect (ASD) is recognised as one of the most common congenital cardiac abnormalities, and is associated with atrial tachyarrhythmias—often the associated morbidity that brings them to the attention of a physician.\textsuperscript{216,217} A meta-analysis has recently shown that ASD closure, either surgical or percutaneous, is associated with a reduction of pre-existing atrial tachyarrhythmias in the short and medium, but not long term.\textsuperscript{218} The predisposing atrial substrate resulting from chronic atrial stretch was described by Morton et al. and Roberts-Thomson et al. as including an increase in the ERP with widespread conduction abnormalities, including regional conduction slowing, areas of local block and electrogram fractionation.\textsuperscript{8,219} There was a reduction of atrial voltage suggesting a loss of atrial myocardium with chronic stretch. In the study by Morton et al, follow-up data in a small subset of patients demonstrated a trend towards a regional reduction in the ERP and an increase in other areas. However there was maintenance of widespread conduction abnormalities seen along the crista terminalis, suggesting this may contribute to the long term continuation of atrial tachyarrhythmias after closure of the septal defect.\textsuperscript{8}
1.3.3.3.7 ATRIAL FIBRILLATION

An abnormal atrial substrate has also been described in patients with ‘lone’ AF in the absence of other obvious contributors. In this study, 25 patients with lone AF were compared to a reference group with supraventricular tachycardia. Patients with AF were found to have a characteristically abnormal substrate with widespread conduction slowing and site-specific delay. ERP was prolonged, in keeping with studies on clinical substrates contributing to atrial remodeling. There was loss of atrial myocardium seen with a reduction in electrogram voltage, however no area of electrical silence suggesting scar was seen. In a further study, a greater numbers of fractionated electrograms in juxtaposition to areas of higher dominant frequencies were observed in patients with persistent compared to paroxysmal AF, when activation mapping was performed during AF. An interesting study by Wong et al. further added to the complexity of the underlying atrial substrate in patients with ‘lone’ AF by identifying direction-dependent abnormalities in patients with AF compared to a reference group. There was greater reduction in signal voltages, slowing in conduction velocity, prolongation of biatrial activation times and an increase in the length and number of lines of conduction block with greater numbers of fractionated electrograms when pacing was performed from the distal coronary sinus compared to sinus rhythm. This suggests perhaps a greater arrhythmogenic potential from ectopics emanating from the left atrium compared to the right.

In human studies assessing reversibility of electrophysiological changes, Yu et al. assessed 19 patients with 6 months of persistent AF
who underwent successful external defibrillation.\textsuperscript{222} When compared to control subjects, baseline studies demonstrated shortening of refractoriness, maladaptation of rate response and slowed conduction, consistent with observations from animal models. There was reversal of these changes, except for atrial conduction, which remained impaired over a 4 day evaluation period. Raitt et al. examined 28 patients before and 1 hour after cardioversion, and then at 19 weeks for those still in sinus rhythm. They found the ERP increased, sinus node function improved, and, an increase in conduction velocity which occurred at heterogeneous rates in different regions of the atrium.\textsuperscript{223}

1.3.3.3.8 PACING

In a pacing study, Sparks et al. demonstrated the adverse effects of loss of AV synchrony induced by VVI pacing, with a non-uniform increase in ERP at 4 atrial sites. These changes were reversed with reestablishment of AV synchrony by DDD pacing.\textsuperscript{224} The benefits of synchronous pacing for AF have also been suggested in cardiac resynchronisation therapy (CRT), such as described by Lellouche et al.\textsuperscript{225} In this study, compared to non-responders, responders to CRT demonstrated a reduction in LA size and significant decrease in the incidence of persistent AF (17\% vs. 2\%). Long term benefits of CRT response were documented at 6 month follow-up with ongoing reduction in the incidence of persistent AF as well as shorter duration of episodes of AF.
1.3.3.3.3.9 PULMONARY VEINS

Given the importance of these structures as previously described above, an abnormal substrate within the pulmonary veins has been documented. Patients with paroxysmal and persistent AF were matched to patients with left-sided accessory pathways and high-density 3D electroanatomic maps of the pulmonary veins were created. These observations determined that the pulmonary veins of patients with AF had lower signal voltages that were more complex, had shorter PV sleeve length, shorter ERPs and reduced conduction velocities. These findings were more notable in persistent AF than paroxysmal disease suggesting an advanced stage of remodeling with maintenance of arrhythmia.

The effect of AF itself on the pulmonary veins, was investigated by Rostock et al. They identified that after exposure to AF, the ERPs of the pulmonary veins were significantly decreased. However, the reduction was more than that seen in the atrial body. Interestingly, conduction slowing was only seen in the pulmonary veins, not the atria, after exposure to short duration AF. These electrophysiological changes after exposure to AF were observed during pacing from the pulmonary veins, which more easily induced AF.

1.3.3.3.4 COMPLEX ATRIAL SIGNALS

Several studies have documented the presence of complex fractionated atrial electrograms (CFAE) and their relationship to adverse remodeling. In this context, the fractionation of the atrial electrogram essentially refers to regions of underlying substrate change, either due to the presence of fibrotic change with wave turning and
breaking due to tissue anisotropy, or slowed conduction. Microstructure computer models have demonstrated that obstacles cause significant changes to the electrogram waveforms recorded and thus the presence of atrial fibrosis can lead to altered wavefront propagation, with resultant collision of signals and fragmentation.

Indeed, these signals have been targeted for ablation. Nademanee et al. described as fractionated electrograms those with 2 or more deflections or continuous activity over a 10 second period, or electrograms with a mean cycle length of <120 ms over a 10 second period. Their eradication resulted in 91% one-year arrhythmia and symptom freedom in a population of 121 patients treated.

Several investigators have advanced knowledge on the aetiology of CFAE’s beyond changes in local myocardial architecture. In this regard, CFAE’s can be defined on the basis of their periodicity on spectral analysis as demonstrated in isolated sheep hearts. This has also been applied to human hearts. Rotor stability results in a change in the power spectra of measured signals outside their immediate vicinity. Hence, increased meandering of the rotors driving the AF resulted in a reduction in activation periodicity and increased fractionation. Further to this, in 24 patients Atienza et al. also concluded that wavefront acceleration (i.e. progressive cycle length shortening, ahead of drifting rotors) was contributory to signal fractionation on the posterior LA wall, reflecting functional deterioration in atrial conduction properties in response to periodic input acceleration. They highlighted the importance of rate dependence and that transitions from organised to fractionated electrograms were always preceded by
progressive cycle length shortening resulting in wave break, wave
direction change and reentry around lines of functional block. This
relationship has been again observed in patients undergoing ablation
whereby pulmonary vein isolation results in AF organisation and a
reduction in the burden of CFAE.\textsuperscript{233} Thus, an interplay between high
frequency sources and underlying myocardial anatomy are likely
responsible for the aetiology of CFAEs.

1.3.3.3.5 CATHETER ABLATION OF ATRIAL FIBRILLATION

Based on the findings of Haissaguerre in 1998\textsuperscript{5}, catheter ablation
has gained acceptance as a therapy for maintenance of sinus rhythm in
patients with AF. Recent guidelines\textsuperscript{234} have changed the classification of
catheter ablation for AF from class IIa to class I with a revision of the
wording to reflect the groups appropriately treated with this modality –
namely symptomatic patients, those with no or mild LA enlargement and
no or mild LV dysfunction. The goals of ablation in paroxysmal AF,
isolation of the pulmonary veins and elimination of non-pulmonary vein
triggers, and its utility has been demonstrated in clinical trials.\textsuperscript{5,235-237}
Given the structural and electrical changes that occur with more
persistent AF,\textsuperscript{142,238} additional substrate ablation is necessary to achieve
and maintain sinus rhythm.\textsuperscript{239} The approach to ablation of persistent
atrial fibrillation remains controversial, but Oral et al. found no additional
benefit for ablation of complex fractionated electrograms over PVI.\textsuperscript{240}
Additional substrate ablation, such as structures annexed to the LA, e.g.
LA appendage and coronary sinus, has been found to be complimentary
allowing termination in 87\% of patients with long-lasting persistent
AF. Nademanee et al. described an approach focusing on CFAE alone, resulting in termination of AF and long term maintenance of sinus rhythm of 76% at one year, and, 91% free of AF after 2 procedures. However, these results have not been obtained by other operators with CFAE ablation alone resulting in success rates as low as 29%.\textsuperscript{239,242}

Induciblity of arrhythmia as an endpoint has been previously addressed by several investigators. Haissaguerre et al. examined the modification of AF cycle length during catheter ablation in 70 patients and found that, AF cycle length prolongation and signal fractionation reduction was greatest in those patients in whom termination of AF was achieved. Additional substrate ablation e.g. linear ablation lead to AF cycle length prolongation and termination of AF, as well as increasing the percentage of patients rendered non-inducible after the ablation.\textsuperscript{243} Oral et al., found that LA circumferential ablation was effective in eliminating AF and with additional substrate ablation of fractionated electrograms, non-inducibility could be achieved. At 6 months there was a trend towards a better AF free outcome in patients rendered non-inducible with additional substrate ablation.\textsuperscript{244}

A Cochrane review of catheter ablation in paroxysmal and persistent AF highlighted the heterogeneity of approaches and the high recurrence rate of AF.\textsuperscript{245} This review, as with other meta-analyses was unable to recommend the best catheter ablation method.\textsuperscript{245-247}
1.3.3.6 MECHANISMS OF STRUCTURAL CHANGE IN AF AND POTENTIAL INTERACTIONS WITH OSA

There is now substantial evidence for an atrial structural substrate, most notably for the role of interstitial fibrosis in promoting conduction slowing and delay with subsequent heterogeneity in electrical impulse propagation. Cardiac fibrosis has been shown to be a remodeling endpoint in a variety of disease states including age, mitral valve disease, cardiac dysfunction, and ischaemia. The structural changes occur more slowly than electrophysiological changes. Experimental models have demonstrated qualitative and quantitative differences in the remodeling observed within the atria and ventricles, with the atria appearing more susceptible. Specific cardiac changes documented in animal experiments, together with observations and biopsies from patients with AF, include enlargement of the atrial chambers, loss of muscular contractility and contractile apparatus, cellular hypertrophy, cellular dedifferentiation, apoptosis and fibrosis.

More recently, delayed-enhancement magnetic resonance imaging (DE-MRI) has been used to detect atrial fibrosis before radiofrequency ablation and further, used as a predictor of procedural outcome. In this study 81 patients prior to their ablation procedure, and 6 healthy volunteers were scanned with MRI. Electroanatomic maps depicting low voltage areas were compared to MRI images of delayed enhancement. After a mean followup of 9.6±3.7 months, 14% of patients with minimal scar burden had a recurrence, compared to 43% and 75% of patients with moderate and extensive enhancement respectively.
(P<0.001). Hence, the degree of remodeling leading to fibrosis may provide an index of disease progression and lend insight into predicting outcomes from ablation.

1.3.3.3.6.1 ATRIAL DILATATION

Atrial dilatation was observed in the Morillo et al. model of sustained AF during chronic rapid pacing in the atrium.\(^{137}\) They observed that an increase in atrial area of 40% was necessary to induce sustained arrhythmia and was also highly correlated to AF inducibility. An increase in LA size with a loss of contractility was also observed in a mitral regurgitation/atrial pacing model conducted by Everett et al.\(^{268}\) Here, conversion from chronic AF to sinus rhythm resulted in electrical remodeling. However, the gross and ultrastructural changes were still present, and facilitated inducibility of arrhythmia, suggesting a significant contribution to AF recurrence. Some studies such as that by Schoonderwoerd et al. demonstrated that fast atrial rates alone may not cause structural changes, but are more dependent on concomitant high ventricular rates causing a hemodynamic overload.\(^ {254,257}\) Similar consequences of morphological dilatation (i.e. atrial “stretch”) were examined in a guinea pig model, where atria were stretched by the inflation of a fluid filled catheter.\(^ {269}\) This demonstrated significant electrophysiological changes on the monophasic action potential coincident with the presence of atrial ectopics or arrhythmia.

In patient studies of AF, atrial dilatation is a notable finding in the presence of identifiable substrate, and in patients with no other causative condition other than AF itself.\(^ {6,9,10,251,262,263}\) Atrial dilatation in the presence of AF and flutter, and its effect on atrial refractory periods and
conduction velocity, was further highlighted by Chen et al., indicating a more notable substrate for AF in the presence of a dilated chamber.\textsuperscript{270} Clinically, vulnerability and longevity of AF in the presence of stretch has been reproduced in several studies on substrates contributing to AF, such as mitral valve disease and hypertension.\textsuperscript{195,197,259,271-273} Additional to electrical remodeling, stretch and dilatation can lead to cellular abnormalities involving both the cardiac cells and extracellular matrix. Hypertrophy of myocytes in response to chronic atrial dilatation has been observed, and appears to have some dependence on the duration of the overload.\textsuperscript{188,272,273} Ausma et al. noted that the degree of cellular hypertrophy over a 9-23 week period of pacing in instrumented goats was up to 195\% of baseline cell size.\textsuperscript{256}

1.3.3.3.6.2 CELLULAR DEDIFFERENTIATION

Cellular dedifferentiation describes the trend towards a more foetal phenotypic profile as perhaps an adaptive mechanism. This has been demonstrated by Rucker-Martin et al. in 24 human RA specimens cultured and analysed immunocytochemically.\textsuperscript{264} In the specimens from fibrillating atria, foetal contractile elements such as smooth muscle $\alpha$-actin and $\beta$-myosin heavy chain antibodies were demonstrated, and these were absent from the control specimens, indicating marked structural and phenotypic alterations of contractile apparatus. In atria from patients with mitral valve disease, atrial volume and pressure overload have been shown to cause a move from $\alpha$ to $\beta$-myosin heavy chain, further indicating that hemodynamic overload can effect the cardiac myosin phenotype.\textsuperscript{274} Besides loss of sarcomeres, other changes include dismantling of the sarcoplasmic reticulum, myolysis,
glycogen accumulation, and mitochondrial changes with homogeneously distributed chromatin similar to that found during embryonic development.\textsuperscript{254,256}

1.3.3.3.6.3 APOPTOSIS

Apoptosis, or programmed cell death, has been observed in tachy-pacing models, and is mainly mediated by proteases known as caspases.\textsuperscript{275} The myolysis is an important component of the loss of contractile function observed.\textsuperscript{276} There appears to be a reliance on the ventricular rate in that no signs of cell death were observed in atria from controlled ventricular rate models.\textsuperscript{254} However, in experimental pacing models designed to caused heart failure, apoptosis is readily observed.\textsuperscript{188,254} In endomyocardial biopsies from the right atrium of patients with lone AF, features of apoptosis and necrosis have been observed.\textsuperscript{251,261}

1.3.3.3.6.4 FIBROSIS

The laying down of dense and disorganised fibrillar collagen (both reparative—replacing degenerating myocardial cells and reactive—expanding the interstitial space between the myocardial cells) has been observed, ultimately disrupting cellular coupling and impairing conduction.\textsuperscript{188,277-280} These mechanisms have been readily demonstrated in several conditions promoting atrial fibrosis including age,\textsuperscript{194} mitral valve disease,\textsuperscript{249} cardiomyopathies,\textsuperscript{250} heart failure,\textsuperscript{281} and myocarditis\textsuperscript{282}. Considerable research in recent years has identified various neurohormonal and cellular fibroproliferative mechanisms thought to be responsible for this important remodeling process. Three
main triggers for atrial fibrosis, the renin-angiotensin-aldosterone system, inflammation and oxidative stress, will be discussed below. Their relevance to the topic of this thesis, OSA, will subsequently be discussed.

1.3.3.6.5 RENIN-ANGIOTENSION-ALDOSTERONE SYSTEM

Considerable work has demonstrated the importance of angiotensin II, not only as a vasoconstrictor with the flow-on effects of left ventricular hypertrophy, which in itself can promote atrial enlargement and stretch, but also by its proinflammatory/profibrotic effects including promotion of reactive oxygen species via nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase). Goette et al. demonstrated that activated extracellular signal regulated kinases (Erk1/Erk2) and MEK1/2 (its activating kinases) as well as angiotensin-converting enzyme (ACE) were elevated in patients with paroxysmal and chronic AF undergoing heart surgery, suggesting an increased activity of intracellular signal transduction pathways in fibrillating atrial tissue. Moreover, the levels of ACE were lower in those patients taking ACE-inhibiting medications. Boldt et al. investigated the role of angiotensin II receptor subtypes in patients with AF or sinus rhythm with and without mitral valve disease. They found that there was upregulation of the subtype 1 receptor in the LA of AF patients but not the RA (without influence of the mitral valve status), suggesting a role for enhanced receptor expression in the pathogenesis of AF.

Transgenic mouse models (ACE 8/8) showing 100-fold increase in cardiac ACE, but no increase in kidney or vascular endothelial ACE levels, demonstrate a high incidence of sudden cardiac death despite
normal ventricular size and function and normal blood pressure. In this model, macroscopic and histological analysis demonstrated atria characterised by enlargement and fibrosis. There was electrocardiographic evidence of heart block. Furthermore, the presence of fibrosis in experimental models of congestive cardiac failure is associated with increased levels of atrial tissue angiotensin II and inhibition of the enzyme partially reduces cellular apoptosis, and thus, consequent atrial remodeling. This manifests as a reduction in atrial fibrosis, conduction time, conduction heterogeneity and mean AF duration.

From a therapeutic viewpoint, valsartan (an angiotensin receptor blocker (ARB)), when given to spontaneously hypertensive rats, reduced interstitial fibrosis, as did candesartan when given to dogs subjected to rapid atrial pacing. There is growing evidence of the significant effect of ACE inhibition from human trials such as TRACE, where patients with reduced LV ejection fraction post myocardial infarction were randomised to the ACE inhibitor trandolopril or placebo. In the ACE inhibitor treated group, Cox regression analysis revealed a significantly reduced risk of developing AF. In a systematic review of 11 studies examining the effects of ACE inhibitors and ARB’s, these medications portended a 28% risk reduction of AF with the greatest benefit observed in those patients with heart failure. Despite these results, however, a recent study examining the effects of valsartan in 1,442 patients with AF revealed that it was not associated with a reduction in the incidence or recurrence of AF. Therefore, further studies on the primary and secondary prevention of AF with this group of medications is required.
In animal experimental models (e.g. a rat model of hyperaldosteronism) spironolactone had anti-fibrotic effects without necessitating the reduction in blood pressure. A study in ventriculally paced dogs with congestive cardiac failure demonstrated beneficial effects of spironolactone, including shortening of conduction times, and a reduction in the inducibility of AF and duration of AF episodes. Similar reports have emerged for eplerenone, a selective mineralocorticoid receptor antagonist.

Mineralocorticoid antagonists have been shown to have significant positive effects on morbidity and mortality (30% reduction in both mortality and hospitalisations) in patients with severe heart failure, as demonstrated in the RALES (Randomized Aldactone Evaluation Study) study. Interestingly, in a sub-analysis of 261 patients from the RALES trial, serum procollagen derivatives were assessed and it was found that in patients taking spironolactone, there was a reduction in the markers of cardiac fibrosis synthesis. Moreover, high procollagen type III amino-terminal peptide levels were associated with an increased risk of death (RR 2.36), which was reduced in the spironolactone group. Also, in a study examining the differences in cardiovascular morbidity in patients with primary aldosteronism and secondary hypertension versus those with essential hypertension, aldosteronism was associated with an odds ratio (OR) of 12.1 for a history of AF, and an OR of 4.2 for previous stroke.

The mechanisms and pathways by which spironolactone exerts its positive effects on the atrial myocardium have not been completely elucidated. Possibilities include a reduction in the expression of
angiotensin 1 (AT) receptors in the heart, as demonstrated in an experiment where aldosterone-salt treatment was seen to cause left ventricular hypertrophy and fibrosis. Treatment with spironolactone prevented aldosterone induced elevation in AT1 receptor density, suggesting that it might be a target for aldosterone. Johar et al. investigated the role of Nox2-NADPH oxidase in aldosterone-induced cardiac fibrosis using Nox2-/- mice compared to wild type. They found that the wild-type animals demonstrated a response characterised by increases in NADPH-oxidase activity, nuclear factor κβ (NF-κβ) and significant interstitial fibrosis. These were inhibited in the knockout mice, suggesting profibrotic effects of aldosterone activating Nox-2 containing NADPH oxidase.

Studies in rats have demonstrated episodic hypoxia can cause an increase in the blood pressure mediated through sympathetic increases to renin-angiotensin-aldosterone system. Intermittent hypoxia has been shown to activate the renin-angiotensin system via AT1 receptor and contribute to an increase in arterial blood pressure in humans. It is plausible, therefore, that these hypoxia induced activation of these pathways contribute to the proinflammatory and profibrotic endpoints leading to atrial remodeling and thus AF.

1.3.3.6.6 TGFβ1/SMAD

Secreted by cardiomyocytes and fibroblasts, transforming growth factor beta 1 (TGFβ1) has been implicated in tissue fibrotic processes affecting many organ systems, most notably in cardiac hypertrophy and heart failure, with its expression related to the degree of fibrotic change. It has both autocrine and paracrine effects i.e. has been
shown to stimulate the production of fibrillar components by cardiac fibroblasts, promote fibroblasts to convert phenotypically to myofibroblasts as well as affecting its own self-amplification in myofibroblasts.\textsuperscript{314-317} Transgenic mice models and experiments blocking the end effects of TGF\textsubscript{B1} signalling, have further demonstrated its role in fibrotic change.\textsuperscript{318,319} There is tight interplay between ATII and TGF\textsubscript{B1}, with upregulation of TGF\textsubscript{B1} mRNA by ATII demonstrated in fibroblasts, myofibroblasts and myocytes including human atrial tissue.\textsuperscript{320-323} Paracrine release of TGF\textsubscript{B1} causes cardiac myocytes to hypertrophy via mediation of AT II systems.\textsuperscript{324} Fibroblast activation and collagen deposition is mediated via an intracellular signalling cascade, of which phosphorylation of the SMAD protein (homolog of the \textit{Drosophila} protein “mothers against decapentaplegic”, MAD, and the \textit{Caenorhabditis. elegans} protein, SMA) is an essential event, resulting in ferrying of signals from the cellular surface to the nucleus.\textsuperscript{325-327}

Studies on MHC-TGFcys33ser transgenic mice, which increased atrial specific fibrosis, when compared to wild type mice, have shown an increased susceptibility to pacing induced AF as well as conduction slowing and conduction heterogeneity, suggesting a central role for TGF\textsubscript{B1} induced changes.\textsuperscript{328} Also, in a pressure-overloaded mouse model, bone monomorphic protein 7 (BMP-7) which is known to inhibit TGF\textsubscript{B1} systems, has been found to reduce cardiac fibrosis by up to 40%, when administered systemically.\textsuperscript{329}

Differences in the fibrotic processes in the atria and ventricle were highlighted in an experimental model of ventricular tachycardia pacing in dogs causing congestive cardiac failure.\textsuperscript{253} In this study, tissue changes
culminating in inflammation and cell death were more notable in the atrial samples, and activated TGFβ₁ was found in increased levels in LA tissue compared with no significant changes in the ventricular myocardium. Lee et al. have demonstrated the ability to reduce the expression of TGFβ₁ leading to a reduction in atrial fibrosis, conduction heterogeneity and ultimately the duration of AF in CHF dogs treated with the anti-fibrotic drug pirfenidone, which has been used in limited clinical trials on other organ systems, including liver fibrosis and pulmonary fibrosis. \(^{330-332}\)

1.3.3.6.7 INFLAMMATION

1.3.3.6.7.1 AF

There is now a well established link between cardiovascular disease and inflammation, with an emphasis on its relationship to atherosclerosis.\(^{333}\) C-reactive protein (CRP), an acute phase reactant produced by the liver, has been established as a sensitive biomarker of systemic inflammation in cardiovascular disease.\(^{334,335}\) This link has been extended to arrhythmia research.

In 1997, Bruiins et al. examined 19 patients undergoing myocardial revascularisation, finding a relationship between post-operative CRP levels and the presence of supraventricular tachyarrhythmias or atrial fibrillation.\(^{336}\) Chung et al. also demonstrated this important association in a study on 131 non-surgical patients. They found that there was a stepwise elevation in CRP dependent on the burden of AF.\(^{337}\) Also, a 1mg/dl increase in serum CRP was found to be associated with a 7 times risk of recurrent AF, with the risk of permanent AF being 12 times greater than control patients.\(^{338}\) In a large population-based cohort from the Cardiovascular Heath Study, Aviles et al.
documented baseline CRP levels in 5,806 subjects over the age of 65, and found that baseline levels of CRP predicted both present AF in those already with the arrhythmia, as well as future development of AF in those without a known history. CRP has also been shown to correlate with LA diameter, and in this study, longer AF duration was associated with CRP elevation and larger LA dimension.

The importance of low CRP levels at baseline was also demonstrated in a study on patients undergoing electrical cardioversion for AF. Low high sensitivity-CRP (hs-CRP) was associated with the maintenance of sinus rhythm at 6 month follow-up. Interestingly, a study on 52 patients with persistent long lasting AF, not only found that hs-CRP was the only independent predictor of AF recurrence, but also that in patients who maintained sinus rhythm, there was a gradual decrease in hs-CRP when compared to their initial evaluation. This suggested that perhaps that AF in itself was a causative agent for the serum levels of CRP.

Subsequent meta-analysis of prospective studies has solidified the finding of an increased CRP association with greater risk of post-cardioversion AF. Furthermore, CRP has been implicated as not only a marker of the inflammatory process, but also as a contributor to myocardial damage. Long chain acylcaritines and lysophosphatidylcholines have been found to be generated from CRP binding to phosphatidylcholine in the presence of Ca\textsuperscript{2+} ions, and this has been shown to lead to cellular membrane dysfunction due to a detrimental affect on transmembrane ion transport.
Similarly, IL-6, a proinflammatory cytokine, has also been shown in human studies to be associated with AF presence, duration and LA diameter as well as postoperative AF. Studies have also demonstrated genetic polymorphisms of IL-6 in patients with postoperative AF and CAD, suggesting perhaps a genetic component to arrhythmia susceptibility.

Histological specimens obtained from patients with lone AF have demonstrated an inflammatory component Frustaci et al. examined endomyocardial biopsies of the RA septum and ventricles in 12 patients with paroxysmal lone AF, finding that lone AF patients had abnormal but heterogeneous atrial histology characterised by cellular hypertrophy, vacuolar degeneration of atrial myocytes, fibrilolysis, lymphomononuclear infiltrates, cellular necrosis and fibrosis. Another study using immunohistochemical and Western blot analysis showed abnormal myocytes with evidence of DNA breakage and decreased expression of antiapoptotic proteins suggesting the fibrillating atria were subject to apoptotic death myocyte death myolysis. Transmural tissue sections derived from animal studies have demonstrated inflammatory infiltrates not seen in comparative control specimens. In a sterile pericarditis canine model, the presence of inflammation was associated with abnormal distribution of Cx40 and Cx43, with absence noted epicardially, and normal distribution endocardially. This was associated with prolonged atrial activation time and abnormal conduction, suggesting a possible role of inflammation induced altered connexins in the aetiology of the structural changes leading to a propensity to AF. Human studies in postoperative AF have demonstrated
the effects of inflammation on atrial electrophysiological properties, reporting a slowing of conduction and conduction inhomogeneity associated with longer duration induced AF with a beneficial effect of antiinflammatories.\textsuperscript{352,353}

1.3.3.3.6.7.2 OSA

Several studies, both in vitro and animal studies, have demonstrated a link between OSA and inflammation such as NF-κB mediated inflammatory pathways. In an in vitro model study by Ryan et al., HeLa cells transfected with reporter constructs and DNA binding assays for the master transcriptional regulators of the inflammatory (NF-κB) and adaptive (HIF-1) pathways, were observed for transcriptional events after exposure to intermittent hypoxia.\textsuperscript{354} These demonstrated that there was selective activation of inflammatory pathways as a result of the hypoxic stimulus, suggesting its importance in OSA. In vivo studies in mice subjected to chronic intermittent hypoxia demonstrated elevated NF-κB activity associated with an elevation in important dependent gene product iNOS (calcium-insensitive nitric oxide synthase), suggesting a molecular mechanism linking OSA induced inflammation to cardiovascular disease.\textsuperscript{355} In another animal model, rats subjected to repeated airway obstruction were found to have a significant influx of leukocytes and rapid endothelial cell activation, suggesting a triggering of an inflammatory response.\textsuperscript{356}

In human studies comparing non-apnoeic patients to those having mild, moderate and severe OSA, genes dependent on NF-κB such as tumour necrosis factor alpha (TNFα) have been found to be elevated, with the level predicted by the degree of intermittent hypoxia (e.g. as
measured by desaturation index). Moreover, there was an independent association between TNFα levels and excessive daytime sleepiness. Another study suggested a reduction in the level of the inflammatory proteins with nasal CPAP. Proinflammatory cytokines such as IL-6 and CRP have variably been found to be elevated in patients with OSA and some have shown a reduction with CPAP therapy. From three randomised trials on CPAP therapy and C-reactive protein levels, only the smaller study found a reduction in this inflammatory marker with CPAP therapy. The two larger studies may not have demonstrated an effect because of the inclusion of patients with cardiovascular co-morbidities, which may have diluted the effect of CPAP on CRP levels.

In summary, inflammation has been associated with AF and may possibly be causative through changes leading to atrial myocyte damage, their apoptosis resulting in loss of atrial mass, and the subsequent development of atrial fibrosis, culminating in proarrhythmic structural remodeling. Activation of these pathways in patients with OSA may provide a plausible link to the development of AF.

1.3.3.6.8 OXIDATIVE STRESS

The cycle of deoxygenation and reoxygenation is thought to resemble ischemia-reperfusion injury, and thus promote the production of reactive oxygen species (ROS) leading to oxidative stress. This has been demonstrated in cell-culture studies, animal models and human studies. This pathway has been shown to lead to systemic inflammation and endothelial dysfunction, and thus is implicated in atherosclerosis, hypertension, myocardial injury,
hyperlipidaemia, obesity, diabetes mellitus,\textsuperscript{376,377,380,381} cognitive impairment,\textsuperscript{382} and arrhythmic risk including atrial fibrillation.\textsuperscript{383} The main contributors to these mechanisms are NADPH-oxidase and nitric oxide (NO) synthase.\textsuperscript{384} As a result of their unpaired electrons, ROS are prone to react chemically.\textsuperscript{385} As molecular oxygen undergoes univalent reduction - whether resulting from mitochondrial respiration or enzymatic systems (e.g. xanthine oxidase endothelial nitric oxide synthase and reduced NADPH oxidase) - the main ROS molecule (superoxide radical $O_2^-$) is generated.\textsuperscript{385,386} Its interaction with other molecules may lead to more potent ROS molecule formation including hydrogen peroxide, hydroxyl radical and lipid peroxides.\textsuperscript{387} In nature, disruption of the tight balance between oxidation and reduction (termed oxidative homoeostasis) results in oxidative stress.\textsuperscript{385} Historically, the most important discoveries in relation to superoxide radicals are that of: Harman et al.,\textsuperscript{388,389} demonstrating the relationship to atherosclerosis and cancer; McCord et al.\textsuperscript{390-392} on the superoxide dismutase enzyme (SOD); and Babior et al.,\textsuperscript{393-395} demonstrating the significance of the superoxide radical as a protective mechanism, and the importance of NADPH. Of specific importance in OSA was the finding of the relevance of ROS to ischaemia/reperfusion injury with the main sources of ROS being xanthine oxidase, damaged mitochondria and inflammatory cells.\textsuperscript{369,396,397}

1.3.3.6.8.1 AF

In pacing models of AF, increased levels of the by-products of oxidative and nitrosative stress,\textsuperscript{398} and oxidative damage to myofibrillar elements, have been demonstrated.\textsuperscript{399} Indeed, gene transcriptional
profiles have demonstrated regulation of gene expression with a shift towards pro-oxidation in AF. Attenuation of the effects of these molecules has been demonstrated with ascorbate which has been shown to decrease peroxynitrate formation in canine models and attenuated the pacing-induced atrial ERP shortening 24-48 hours after pacing. When supplemented orally to patients undergoing coronary artery bypass grafting, it reduced the incidence of postoperative AF in humans. Reactive oxygen species (ROS) associated with ischaemia-reperfusion induced ventricular arrhythmia can be reduced with Vitamin E analogues and other ROS scavengers, with beneficial effects.

Potential mechanisms have been described by which oxidative stress itself has been shown to modulate pathways which may be contributory to the development of myocardial fibrosis. Important pathways leading to this endpoint include TGF-β1 and mitogen-activated protein kinase (MAPK) subfamilies. Additional to potential influences on prothrombotic pathways, oxidative stress may also alter Ca²⁺ homeostasis via alteration of gating properties of ion channels and transporters (e.g. decreasing the L-type ion channel current) thus contributing to electrical remodeling.

1.3.3.3.6.8.2 OSA

Experimentally, ROS has been documented as a critical component to the pathophysiology of OSA, as they have been detected in the cells, urine and exhaled air of patients, and their levels modulated by therapies for OSA such as nasal CPAP. ROS have been implicated in many associated comorbidities such as hypertension, hyperlipidaemia, obesity and diabetes mellitus. Important signalling
pathways include the MAPK, the nuclear transcription factor nuclear factor (NF-κB), activator protein (AP-1), hypoxia-inducible factor (HIF-1α), sterol regulatory element binding proteins (SREBP’s), and GATA-4. Well studied, but still not completely elucidated, is the role of NF-κB in the production of adipokines, cytokines and adhesion molecules, all of which have important roles in inflammation and atherosclerosis and are thus implicated in the metabolic syndrome and obesity associated with sleep apnoea. Similarly, HIF-1α has a role in neutrophil survival, and is critical to the molecular mechanism involved in the chemoreceptor-mediated excitation of the sympathetic nervous system seen with chronic intermittent hypoxia resulting from OSA.

1.3.3.6.9 ENDOTHELIAL DYSFUNCTION

1.3.3.6.9.1 AF

Endothelial function, an independent predictor of adverse cardiovascular outcomes, is maintained by the production of nitric oxide (NO), with downstream anti-proliferative effects on smooth muscle, anti-adhesion effects on platelets and leucocytes. Endothelial dysfunction may lead to many prothrombotic and proinflammatory effects. Circulating markers, such as Von Willebrand factor (vWF), an acute phase reactant released by the endothelium in response to endothelial damage, promoting platelet adhesion, has been found in high levels in the plasma of patients with AF. Elevated vWF is significantly expressed by left atrial appendage tissue from patients undergoing coronary artery bypass grafting, predicting post-operative AF. This points towards a potential role for endothelial
damage in the complex pathophysiology causing AF. Non-invasive studies examining flow-mediated dilatation,\textsuperscript{431} and more invasive techniques have been employed to assess endothelial dysfunction. Using a Doppler wire one group demonstrated impaired myocardial perfusion, suggesting localised micro-vascular endothelial dysfunction.\textsuperscript{433} Forearm blood flow measurements after intra-arterial acetylcholine and nitroglycerine are impaired in AF, with some augmentation of endothelium-dependent vasodilatation with restoration of sinus rhythm.\textsuperscript{434} Impaired myocardial blood flow in AF has also been shown with positron emission tomography, with some improvement after restoration of sinus rhythm.\textsuperscript{435}

1.3.3.3.6.9.2 OSA

Endothelial dysfunction has been examined in patients with OSA, using forearm blood flow and the response to acetylcholine infusion as a surrogate for endothelial function.\textsuperscript{436} Carlson et al. and others have found that there is a reduction in endothelium-dependent vascular relaxation in patients with OSA, compared to normal and hypertensive patients.\textsuperscript{437,438} Moreover, impairment in endothelial-dependent vasodilatation has been shown to correlate with endothelial cell apoptosis, with CPAP causing a decline in circulating apoptotic endothelial cells, providing an additional mechanism predisposing to vascular injury.\textsuperscript{439}

Further supportive evidence for NO involvement in long term vascular remodeling is found in studies demonstrating suppression of circulating NO in OSA, which is promptly reversed by nasal CPAP.\textsuperscript{440} Overlap can be seen between oxidative stress and endothelial
dysfunction given superoxide generates peroxynitrite, a powerful oxidant, from endothelial NO, which, in turn, interacts with tyrosine residues producing nitrotyrosine. This has been shown to be associated with endothelial dysfunction as demonstrated in work on cultured porcine pulmonary artery endothelial cell monolayers.441 This has been further demonstrated in human studies, where freshly harvested venous endothelial cell expression of nitrotyrosine was shown to be enhanced in OSA patients compared to non-apnoeics, and ameliorated with treatment by CPAP.442 Interestingly, a randomised double blinded trial of allopurinol in moderate-severe OSA patients, measuring flow-mediated dilatation and plasma malondialdehyde levels to assess oxidative stress, demonstrated a consistent reduction in oxidative stress and improvement in endothelial dysfunction, suggesting xanthine oxidase is a contributor to the impaired vasodilatation observed in these individuals.443

Ultimately, oxidative stress leads to inflammation, endothelial dysfunction and atherosclerosis, all key features of the pathophysiology associated with OSA and cardiovascular morbidity.

1.3.3.3.7 AUTONOMIC NERVOUS SYSTEM

1.3.3.3.7.1 RELEVANCE TO AF

AF triggering has been shown to be increased by both vagal and sympathetic mediated mechanisms.444,445 Indeed, heart rate variability studies have shown either vagal or sympathetic dependent mechanisms prior to the onset of AF. 446,447

For most patients without organic heart disease, the parasympathetic nervous system (PNS) appears prominent, with vagal
dependence in AF (first noted by Coumel et al.) well documented. This has been further established through observations of the relationship between arrhythmogenesis and parasympathetic predominance in sleep. A role for the PNS has also been observed in both elite and non-elite athletes. In contrast, in those with structural heart disease, evidence suggests episodes of paroxysmal AF are driven by the sympathetic arm of the ANS. This has also been observed in patients with post-operative AF episodes.

1.3.3.3.7.2 ANATOMY

Extrinsic to the heart, the autonomic nervous system (ANS) consists of brain nuclei, ganglia along the spinal cord and their axons terminating in the heart. Cardiac sympathetic innervation (superior, middle and inferior cardiac nerves) extends from ganglia situated along the cervical and thoracic vertebrae (C1-3, C7-8, T1-7). Superior, inferior and middle branches of the vagus nerve provide the parasympathetic innervation of the heart. Intrinsic to the heart there is a network of autonomic ganglia and axons known as ganglionated plexi which are embedded within epicardial fat pads on both atria and ventricles. The plexi are located on the superior surfaces of the right and left atria, posterior surface of the RA, posteromedial surface of the LA and the inferior and lateral aspects of the posterior LA. There are estimated to be over 14,000 neurons in the human heart, and the PV-left atrial junction is rich in both parasympathetic and sympathetic nerve endings.
1.3.3.7.3 PARASYMPATHETIC NERVOUS SYSTEM

1.3.3.7.3.1 AF

A relationship between triggering of AF paroxysms and ANS modulation of the myocardium, has been long observed.\textsuperscript{75} Parasympathetic innervation to the heart, and in particular, its heterogeneity, has been known since the 1950’s.\textsuperscript{457,458} Muscarine sensitive inwardly rectifying K+ channels ($I_{K_{Ach}}$) coupling directly with G protein are found in the sinoatrial node and atrial tissue, are the targets for acetylcholine,\textsuperscript{459} and selective blockade of these receptors with NTC-801 has been found to have AF terminating effects.\textsuperscript{460} In areas of high expression of $I_{K_{Ach}}$, such as the posterior LA, there is more notable shortening of the APD, perhaps explaining in part its role in AF initiation and maintenance.\textsuperscript{461}

In the presence of acetylcholine, single extrastimuli delivered after a drive train in canine myocardium, may cause sufficient reduction in ERP to produce reentry circuits, allowing the sustenance of AF.\textsuperscript{462} Animal models have demonstrated the presence of autonomic ganglia evoked rapid ectopic beats inducing AF, which can be overcome with pharmacological sympathetic and parasympathetic blockade.\textsuperscript{463} Furthermore, Jalife et al., in a Langendorff-perfused sheep model of rapid pacing in the presence of acetylcholine, identified stable sites of highest dominant frequency in the RA and LA using bipolar and optical mapping techniques.\textsuperscript{92} Spatiotemporal periodicity was seen in the LA during all the episodes recorded, the majority having a single site with periodic activity at the highest dominant frequency and this was often localised to the posterior LA near or at the ostium of the pulmonary vein.
1.3.3.3.7.3.2 OSA

In a recent study described in a previous section 17 pigs were intubated with negative tracheal pressure applied intermittently to simulate OSA. Obstructive events resulted in a reduction in the ERP and the changes were prevented by the use of atropine or vagotomy, suggesting that the mechanisms were clearly vagal mediated. From a cellular perspective, only combined blockade of the $I_{kr}$ and $I_{kur}/I_{to}$ channels (using sotalol and AVE0118) resulted in attenuation of the negative tracheal pressure induced ERP shortening and AF inducibility. Neither drug alone produced the desired endpoint, in keeping with previous studies demonstrating the inability of $I_{Kur}$ alone to terminate cholinergic AF.

1.3.3.3.7.4 SYMPATHETIC NERVOUS SYSTEM
1.3.3.3.7.4.1 AF

Adrenergic effects differ from vagal stimulation, with sympathetic nerve stimulation appearing less effective in promoting AF. In 1930, Andrus et al. infused epinephrine in atropine-treated dogs and found a reduction in the atrial ERP by up to 40%. However, unlike in the presence of vagal stimulation, the introduction of single stimuli was not able to induce AF in the presence of sympathetic stimulation. Rensma et al., in a conscious dog model, examined the effect of various agents on ERP and conduction velocity, finding that isoproterenol only marginally shortened the refractory period and had no effect on conduction velocity, whereas propranolol had no effect on any of the electrophysiological variables. Liu et al. again demonstrated the marked differences in effects finding that parasympathetic stimulation caused marked
heterogeneity in refractoriness, whereas sympathetic stimulation did not.\textsuperscript{444} Also, with simultaneous recording, conduction was relatively organised during sympathetic stimulation, but under parasympathetic stimulation there was marked heterogeneity in conduction displaying areas of functional reentry, suggesting a more obvious substrate for AF.

In heart failure, a dynamic substrate for atrial fibrillation has been recently described with autonomic remodeling in the LA and PV’s.\textsuperscript{466} In this work, electrophysiological mapping was conducted in a ventricularly-paced dog model of heart failure. Various manoeuvres were performed to stimulate or block either or both arms of the ANS. Atria were examined for nerve density and distribution as well as receptor densities. With congestive heart failure, there was a marked increase in the number of sympathetic fibrils and parasympathetic fibres, bundle size, cardiac ganglia, and sympathetic receptor density. A marked increase in the effect of sympathetic stimulation on atrial electrophysiology was also demonstrated.

1.3.3.3.7.4.2 OSA

Several studies in animals and humans have demonstrated the relationship between OSA and sympathetic nervous system (SNS) activation (this being attributed to the intermittent hypoxia and effect of recurrent arousals), with much of the interest directed towards the blood pressure surges and persistently elevated daytime blood pressure record in OSA patients. Animal data from experiments on rats exposed to intermittent hypoxia up to one month have demonstrated elevated blood pressure that could be prevented by surgical denervation of peripheral chemoreceptors, as well as chemical denervation of the PNS with
Putative mechanisms may involve chronic intermittent hypoxia causing hypoxia evoked oxidative stress via HIF-1 activation and HIF-2 down-regulation, leading to acute oxygen hypersensitivity in the carotid bodies and adrenal medullary chromaffin cells.468

This has been further demonstrated in feline model examining carotid body chemoreceptors.469 Chronic intermittent hypoxia was found to selectively enhance carotid body ventilatory responses to hypoxia with higher basal discharges and response to acute hypoxia. There was also a marked change in the power spectral distribution in heart rate variability, suggesting early changes to autonomic activity in favour of sympathetic augmentation.

Several studies in humans have shown an increase in SNS outflow in OSA, as measured by sympathetic activity in muscles and plasma and urine levels of catecholamines. Somers et al. demonstrated that sufferers of OSA had high levels of peripheral nerve activity even when awake, and that this could be ameliorated by CPAP.470 They also showed that peak sympathetic activity occurred over the last 10 seconds of each apnoea, increasing up to 299+/−96% during sleep stage II. This was also associated with a surge in the blood pressure.470 Indeed, sympathetic stimulation and resultant nocturnal hypertension has been implicated in the non-dipping pattern of blood pressure change seen in OSA sufferers.13,471,472

Heart rate variability studies on individuals with OSA have been performed by many groups, demonstrating various changes on indices measured in the time and frequency domains, as well as more complex
non-linear measurements such as entropy.\textsuperscript{473-476} There is robust literature on altered heart rate variability and its prognostic relationship to cardiac events and mortality.\textsuperscript{477} In short, the frequency domain demonstrates two discrete frequency bands with physiological correlates - the low frequency (LF 0.04-0.15 Hz), indicating sympathetic activity predominantly (although controversy exists in the literature, with some interpreting the parameter to have both sympathetic and parasympathetic influences),\textsuperscript{478} and the high frequency (HF 0.15-0.4 Hz), being more an indicator of vagal activity.\textsuperscript{479} Shiomi et al. studied 12 patients with severe OSA, and found interesting changes in the very low frequency peak (VLF \(\leq 0.04\) Hz).\textsuperscript{479} Here, the heart rate variability increased synchronously with periods of hypoxia (i.e. absent air exchange I, a cycle length of approximately 25-120 seconds). The implication of this finding is that detection of changes in this frequency can be used for detecting OSA from 24hr Holter recordings. An early study by Guilleminault et al. using cyclical variations in heart rate variability to screen for OSA on 24 hour Holter examined 400 patients, finding that changes in the HF zone were attributable to bursts of parasympathetic activity, and could be blocked by atropine sulphate, eliminating the bradycardic component.\textsuperscript{480} Additionally, others have demonstrated increases in the VLF, LF and the LF/HF ratio with a decrease in the HF, highlighting the importance of dominant sympathetic activation.\textsuperscript{481} Other potentially important measures include heart rate turbulence (HRT), based on assessment of turbulence onset, and slope, analysed from RR intervals after a single ventricular premature beat. With ANS abnormalities, the usual rapid change in heart rate seen in
response to a ventricular ectopic beat is lost. Several groups have demonstrated the relationship between OSA measures and HRT, suggesting sympathetic activation at night and baroreflex dysfunction.482-484

Arousals and their effects on blood pressure and sympathetic outflow have been demonstrated in both animal and human models. In a model where 6 dogs were intubated via a chronic tracheostomy (to allow mechanical breathing and abolish the effects of spontaneous breathing on lung volumes and blood gases), Homer et al. examined the effects of arousal on NREM sleep. They documented that arousal from NREM sleep (as compared to relaxed wakefulness) was associated with acute activation of the SNS and withdrawal of the PNS, the effects of which could be abolished by blocking both limbs of the autonomic system.485

Similarly in humans, Somers et al. measured blood pressure, heart rate and sympathetic outflow using microneurography, providing a direct measurement of efferent sympathetic-nerve activity related to muscles and blood vessels in normal individuals during wakefulness, and through the stages of sleep. The findings included observations that arousals in stage 2 resulted in evidence of sympathetic nervous activation, including high-amplitude deflections on the electroencephalogram (K complexes), transient increase in blood pressure and bursts of muscle sympathetic outflow.486

In an experiment on humans using the Mueller manoeuvre to simulate the effect of apnoeas and inspiratory effort on intrathoracic pressure in OSA, Sommers et al documented altered hemodynamics during acute closure of the upper airway with persistent inspiratory
effort, in addition to hypoxia and arousals. As with clinical OSA, the negative intrathoracic pressure generated as a result of the pharyngeal occlusion lead to an increase in the left ventricular transmural pressure.\textsuperscript{487,488} Oscillation in the blood pressure and sympathetic nervous activation was clearly demonstrated, with a >200% surge in sympathetic outflow and 14% increase in blood pressure noted at the end of apnoea.\textsuperscript{489}

1.3.3.7.5 GANGLIONATED PLEXI

1.3.3.7.5.1 AF

A study by Hou et al. sought to determine the interaction between the extrinsic and intrinsic ANS.\textsuperscript{490} In dogs, atrial electrophysiology following unilateral vagosympathetic trunk stimulation during AF was compared before and after sequential ablation of the anterior right, inferior right, and superior left ganglionated plexi. Varying effects on the sinus rate, ventricular rate, ERP and AF inducibility were documented, and thus the ganglionated plexi functioned as intricate “integration centres” modulating the autonomic interactions between the extrinsic and intrinsic cardiac ANS. In animal models of atrial tachycardia remodeling, with ablation of these structures resulting in a prolongation of the atrial ERP and reduction in the susceptibility to AF.\textsuperscript{491,492}

In one study on patients with paroxysmal AF, ganglion ablation, when added to pulmonary vein isolation, resulted in an increase of the 12 month arrhythmia free rates from 60.6% to 85.3%.\textsuperscript{493} In another study on 70 patients with paroxysmal AF, randomised to either anatomic ganglionated plexi ablation or circumferential pulmonary vein isolation, anatomic ganglionated plexi ablation was an independent risk factor for
late recurrence (HR 2.08), suggesting ablation of these structures alone is not sufficient.\textsuperscript{494}

1.3.3.3.7.5.2 OSA

An important study by Ghias et al. promoted further insight into the importance of these plexi, with particular reference to OSA. They developed an experimental model of simulated OSA to determine the underlying autonomic mechanisms and the consequential effect of ganglionated plexi ablation, as a method of preventing AF in the presence of apnoea.\textsuperscript{495,496} Here, in 30 anaesthetised dogs, neural recordings were made from the anterior right ganglionated plexi after thoracotomy. Apnoea was induced by closing off the ventilator during end expiration, resulting in progressive firing of the ganglionated plexi that was associated with a shortening of the atrial ERP, slowing of the heart rate and an increase in the systolic blood pressure. Fat pad ablation destroying this ganglionated plexi inhibited the occurrence of apnoea-induced AF, even during more prolonged apneic episodes. In four dogs within the study, autonomic blockade was delivered via an infusion of beta-blockers and atropine. This resulted in an inability to induce AF during an apneic period of 2.5 mins, further suggesting a role of the ANS in OSA mediated AF

1.3.3.3.7.6 PV-LA JUNCTION

In recent work on dogs, both parasympathetic and sympathetic blockade were able to suppress pulmonary vein (PV) firing, additionally, both an enhanced Ca\textsuperscript{2+} transient current (blockable by ryanodine) and increased Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange may be required for the induction of arrhythmia.\textsuperscript{497,498} In rabbits, the development of pacemaker activity in the
PVs was attenuated by Ca$^{2+}$ depletion and blockade of the Na$^+$/Ca$^{2+}$ exchanger, and potentiated by beta adrenergic stimulation. This was seen in Langendorff-perfused canine LA studies, which showed the importance of intracellular calcium elevation prior to the onset of focal discharges at the PV-LA junction. In the same study, anatomical importance of the PV-LA junction was seen with abrupt changes in myocardial fibre orientation and increased interstitial fibrosis in the pulmonary veins and PV-LA junction. During pacing induced reentry, phase singularities clustered at the PV-LA junction suggesting these sites to be of increased anisotropy, and hence important for the establishment of arrhythmia. Together, focal discharges and reentry are important aspects of PV triggered activity. This study also demonstrated periodic-acid-Schiff staining of large cells long the endocardium of the PV muscle sleeves. In a study by Tan et al. these same cells were identified at the sites of PV ectopy, in association with a high density of sympathetic nerve endings.

1.3.3.7.7 SUMMARY

Both arms of the ANS have significant effects on atrial electrophysiology. However, these effects have not been fully elucidated and clearly are complex. Modification of the cardiac inputs, such as apnoea related triggering, may have important therapeutic implications on the treatment of atrial fibrillation with ablation. Indeed treatment of OSA with CPAP may effectively abolish adverse neural inputs altogether.
1.4 OBSTRUCTIVE SLEEP APNOEA

1.4.1 HISTORY OF OBSTRUCTIVE SLEEP APNOEA

Whilst sleep apnoea has been recognised as far back as the 4th Century BC, reports only started to appear in the literature just over 40 year ago, when Henri Gastaut documenting polysomnographic features of apnoea in a group of Pickwickian patients. Prior to this, in 1905, Dr William Osler made an interesting reference, in his textbook “The Principles and Practice of Medicine”, to case reports of Dr Christopher Heath’s observations of patients resembling the severely obese, hypersomnolent boy named Joe in Charles Dickens’ 1836 novel ‘The Posthumous Papers of the Pickwick Club’. Periodic breathing was first observed after the mid 1800’s by British physicians reporting on their observations of obstructed breathing, due to glottic obstruction associated with cyanosis during sleep.

The first continuous overnight EEG sleep recordings were published in 1937 and recognised various overnight phenomena such as sleep fragmentations. As the science developed, Dement and Kleitman proposed the first classification based on rapid eye movement (REM) non-rapid eye movement (NREM) sleep periods alternating in cycles throughout the night. It was Dr Henri Gastaut’s descriptions that lead to the coining of “Gastaut Syndrome” (characterised by upper airway obstruction). With additional information from diaphragmatic and respiratory muscle electromyograms, arterial blood gas analysis and observations of ventilation during sleep, upper airway obstruction was
shown to be the cause of sleep apnoea in these ‘Pickwickian’ patients (i.e. the first documented links between obesity and OSA).  

In 1978, Remmers et al. published on the pathogenesis of upper airway occlusion during sleep, focusing on the anatomical and neurophysiologic components leading to the “balances of forces” concept. Further sleep research demonstrated that recurrent awakenings lead to reduced sleep time at night and daytime hypersomnolence that was relieved by tracheostomy. In 1981, a landmark paper published by Sullivan at al. outlined the treatment of obstructive sleep apnoea (OSA), by instituting a pneumatic splint using continuous positive airways pressure (CPAP) via a nasal mask in 5 patients. Using a range of low-level pressure from 4.5-10 cm H\textsubscript{2}O, upper airway obstruction was prevented, allowing the subjects to sleep an entire night without interruption.

These developments set the scene for an explosion of basic scientific, clinical and epidemiological studies documenting the features and pathophysiologic consequences of OSA, and specifically, with regard to the myriad of cardiovascular disease associated with its pathophysiology. This body of work is complementary, adding to our knowledge on OSA and its cardiac interactions causing AF.

1.4.2 EPIDEMIOLOGY OF OBSTRUCTIVE SLEEP APNOEA

The epidemiological features of OSA have been established via several large scale epidemiological studies conducted in the U.S, Europe, Australia and Asia. However, various measurement techniques (e.g. supervised laboratory polysomnography or home monitoring) have been used, and definitions have not been consistent
across the studies. The spectrum of the condition is wide, under-diagnosis is common and the importance of the disease is typified by its associated cardiovascular morbidity.\textsuperscript{522-524}

Pathological daytime sleepiness, a result of the sleep fragmentation resulting from repeated arousals, is well recognized as a cardinal feature of OSA and treatment with CPAP has been shown to alleviate this symptom.\textsuperscript{525-527} The Epworth Sleepiness Scale (ESS), devised in 1991\textsuperscript{528} is a reproducible, easily instituted questionnaire to assess global level of pathological sleepiness,\textsuperscript{529} independent of short-term variations\textsuperscript{530}. In the Sleep Heart Health Study, there was a significant and progressive increase in measures of sleepiness such as the ESS score with increasing apnoea-hypopnoea index (AHI; the number of apnoeas and hypopnoeas per hour of sleep).\textsuperscript{510,531} The percentage with excessive sleepiness defined by an ESS $\geq 11$, independent of factors such as age, BMI and sex, increased from 21\% in subjects with AHI $<5$ (no OSA), to 35\% in individuals with AHI $\geq 30$ (severe OSA). Despite this association, the majority of subjects identified to have OSA on the basis of AHI $\geq 5$ did not report excessive sleepiness. Furthermore, differences in the frequency of arousals did not explain the variation in sleepiness observed. This lack of association with objective measures of sleep extends to multiple sleep latency testing, considered a ‘gold standard’ for excessive daytime sleepiness.\textsuperscript{532} In the Wisconsin sleep cohort, a study using in-lab polysomnography and a two stage sampling procedure (initial questionnaire screening a large population followed by a random selection that undergo more vigorous diagnostic testing), 602 adults aged 30-60 years underwent detailed laboratory
The prevalence of OSA, diagnosed on the basis of AHI \( \geq 5 \), was 9% for women and 24% for men. The syndrome of daytime sleepiness associated with OSA (OSAS) was diagnosed in 2% of women and 4% of men. The Pennsylvania cohort comprised 1,741 patients that had laboratory based polysomnography evaluation for sleep apnoea. Based on an AHI \( \geq 10 \), and the presence of daytime symptoms, the prevalence was 3.9% in men, and 1.2% in women. In an Australian study using home monitoring to measure OSA in 294 Australian men aged 40-65 years from a volunteer register of the Busselton Health Survey, OSAS defined as AHI \( \geq 5 \) and daytime sleepiness, was identified in 3.1% of the studied population. The Spanish cohort, consisting of people aged 30-70 years of age from Vitoria-Gastiez, Basque County, Spain, conducted laboratory based polysomnography and found a much higher prevalence than other studies - 26.2% in men, and 28% in women. The results from this study were probably higher due to the inclusions of ‘arousals’ to signify hypopnoeas.

Studies have also documented differences in the epidemiology of the disease amongst races. Here, the respiratory disturbance index (RDI; similar to the AHI but including all other phenomena causing arousals from sleep) was found to be higher in African Americans, and a significant interaction was observed between age and RDI suggesting young African-Americans were at increased risk for sleep apnoea. This was again observed in a study comparing the elderly population, which found that race was associated with the presence of OSA, independent of sex, age and BMI. More recent studies from Asia, using similar
methods, have shown similar prevalence to the white population - from Hong Kong, 4.1% for males and 2.1% for females,\textsuperscript{515,516} from Mumbai, a study on middle aged Indian males demonstrated an OSA prevalence of 19.5% and OSAS of 7.5%,\textsuperscript{520} and in a Korean population, OSAS prevalence was 2% for males and 3.5% for females.\textsuperscript{519}

The incidence of OSA can be estimated from 3 main population-based longitudinal studies. In the Cleveland Family Study,\textsuperscript{536} for the 286 subjects without polysomnographic evidence of OSA (AHI<5) at baseline, the overall 5-year incidence of OSA was 7.5% for at least moderate OSA or worse (AHI >15), and 16% for an AHI >10, with 63% of study subjects not developing the condition. The mean AHI at baseline was 2.0±1.4 and, after 5 years, was 6.2±7.9. The positive predictors for developing the disease were age (OR per 10-year increase, 1.79), BMI (OR per 1-unit increase, 1.14), sex (OR 4.12 for men vs. women), waist-hip ratio (OR per 0.1 unit increase, 1.61) and serum cholesterol concentration (OR 1.11 per 0.25-mmol/L increase). In the Wisconsin study,\textsuperscript{537} at baseline, 554 subjects had an AHI below 5. Over a 4-year follow-up period, OSA (AHI ≥5) developed in 10.6%. Over an 8-year period the mean AHI increased by 2.6 events/hour, from 2.5 at baseline to 5.1 at follow-up.\textsuperscript{537} In the Sleep Heart Health Study, a large population-based prospective study conducted from July 1989 to January 2000, enrolling 6,441 participants over the age of 40, the incidence over 5 years for those with an AHI<5 at baseline for developing OSA (AHI ≥ 5) was 11.1 % for men and 4.9% for women.\textsuperscript{538}

Several risk factors for the development of OSA have been identified including body mass index, age, gender, BMI, menopause,
smoking, alcohol and genetic predisposition. Craniofacial features and upper airway anatomical predictors will be discussed in the section on pathophysiology of OSA.

The first large population study to include an older group of people was that by Ancoli-Israel et al., where 427 community dwelling Californian men and women aged between 65-95 underwent home-based polysomnography. Of these, 62% had a RDI ≥10 (70% for men and 56% for women). This higher prevalence of SDB in the elderly compared to middle aged adults has been confirmed in several other population-based cohorts. In the Pennsylvania cohort, there was an increase in the prevalence of OSA within the three compared broad age bands—20-44 (3.2%), 45-64 (11.3%) and 65-100 (18.1%). For RDI ≥5, there was a marked increase with age, with up 30% of patients in the 65-100 year old band having some evidence of SDB on polysomnography. Women were analysed separately and also showed an age dependent increase in prevalence - 20-44 (0.6%), 45-64 (2.0%) and 65-100 (7.0%). An even larger difference was seen between middle and older age groups in the Vitoria-Gastiez Spain Cohort, where, for the age group 30-70 the prevalence for AHI ≥5 was 26% for men and 28% for women, and for AHI ≥ 5, 14% for men and 7 % for women; by comparison, in the age group 71-100 group presented at the World Conference in 2001 by Durán et al., for an AHI ≥ 5, the prevalence was 80% in men and 81% in women and for AHI ≥ 15, 57% for men and 49% for women – that is, three to four times higher.

In the Sleep Heart Health Study a 10-year age increment was associated with an increase in the odds of having an AHI ≥15 by 24%.541
This study and others have demonstrated that the prevalence of OSA increases with age until 60, where, thereafter, it reaches a plateau in which the prevalence of moderate to severe OSA remains constant.\textsuperscript{513,541} Interestingly, with the increased prevalence in the elderly population of OSA, there is not an associated increase in the presence of related symptoms, i.e. the OSA syndrome.\textsuperscript{511} Various explanations have been advanced to explain the increased prevalence with age including anatomical changes to the pharynx and pharyngeal surrounds as well as lengthening of the soft palate.\textsuperscript{542,543} Changes to NREM slow wave sleep occur with normal ageing (particularly seen in older men). These changes have been shown to result in increased instability in sleep as well as increased resistance in the upper airways.\textsuperscript{544-546}

Male gender features heavily in both the referrals for clinical evaluation (where the ratio of men to women is about 5-8:1) and in the prevalence of OSA (2-3x greater risk for men compared to women).\textsuperscript{510,512,514,547} In clinical studies rather than population studies, there may be a referral bias favouring men, given females do not tend to present with the typical symptoms of the syndrome (loud snoring, nocturnal gasping and witnessed apnoeas).\textsuperscript{548,549} There also appears to be a gender disparity within different bands of OSA severity, as O’Connor et al. demonstrated in a retrospective review where male-to-female ratio was 3.2:1 for all OSA patients, 2.2:1 for patients with mild OSA (AHI >15) and increased to 7.9:1 for those with severe OSA (AHI >30).\textsuperscript{550} Both anatomical differences and physiological properties and responses of the airways have been advanced to explain the differences.\textsuperscript{551-554} Differences also exist in the polysomnographic
patterns seen, in that women have a tendency for a lower AHI during NREM sleep but are similar to males in REM sleep. Moreover, observed events are shorter and often associated with less oxyhaemoglobin desaturation than men.\textsuperscript{555}

Interestingly, in menopausal women, an increased prevalence of OSA has been observed independent of other risk factors such as age and body habitus, with the odds ratios for AHI ≥15 1.1 with perimenopause and 3.5 with post-menopause.\textsuperscript{556} An analysis of 2,852 women aged greater than 50 years from the Sleep Heart Health Study showed, on multivariate analysis, an OR of 0.55 for OSA (AHI ≥15) for women on hormone replacement therapy, suggesting its beneficial effect on the disease.\textsuperscript{557} It is proposed that female hormones may have an effect on upper airway dilator muscle activity as suggested in a study by Popovic et al. which examined awake genioglossus electromyograms and upper airway resistance in pre- and postmenopausal women.\textsuperscript{558} There are no double-blinded, placebo controlled trials demonstrating a positive effect of hormone replacement therapy on OSA in postmenopausal women. One study has found that women with OSA, when compared to men, have a higher 5 year mortality.\textsuperscript{559}

Obesity is considered a major risk factor for OSA and there is a very tight relationship between the two conditions.\textsuperscript{510,538} In the Sleep Heart Health Study, for individuals with a BMI ≥31 kg/m\textsuperscript{2}, 26% had an AHI >15 and 60% an AHI >5.\textsuperscript{510} Also, for every 1-SD increment in BMI, the OR for OSA (AHI ≥5) was 4.17. Other measures of obesity demonstrated similar increases in the OR (e.g. waist:hip ratio 1 SD increment leading to a 3.41 increase in OR). In the morbidly obese with
BMI >40 kg/m², an AHI ≥5 (i.e. some degree of OSA) was present in 98% of subjects and 33% had AHI ≥65 (severe OSA).⁵⁴¹ Amongst patients who are candidates for bariatric surgery, the prevalence of OSA is reported to be 70-77%, independent of having symptoms suggestive of the disease or not.⁵⁶⁰ Indeed, in one study, the preoperative diagnosis of OSA was already established in 15% of patients, however after routine polysomnography, a further 63% of the cohort were diagnosed with the disorder.⁵⁶¹-⁵⁶³

In the Wisconsin study, an increase in weight of 10% was associated with an increase in the AHI of 32%, and a reduction in weight by 10% caused the AHI to fall by 26%.⁵⁶³ The relationship between OSA and obesity extends to both children and adolescents.⁵¹⁰,⁵⁶⁴ Moreover, both weight gain and weight loss has been shown to be associated with progression and regression of OSA respectively.⁵³⁸ The odds of progressing from mild OSA (AHI<15) to moderate OSA (AHI ≥ 5) with an increase in weight gain of 10% is 6 fold,⁵³⁷ and similarly, studies on patients undergoing bariatric surgery for the treatment of obesity have demonstrated significant improvements in measures of OSA with weight loss.⁵⁶⁵ Interestingly, in this study on patients undergoing weight loss post surgery, after adjustment for weight change and baseline central obesity, there was also a reduction in diabetes and hypertriglyceridemia suggesting far reaching benefits from the amelioration of sleep apnoea, beyond the weight loss alone.⁵⁶⁵

Although the relationship between OSA and obesity is complex, suggested mechanisms involve extraluminal fat deposition (especially posterolateral to the oropharyngeal airspace) resulting in smaller lumen
and increased collapsibility, and truncal obesity leading to a reduction in chest compliance and functional residual capacity. Craniofacial anatomical variants identified to be important in determining the presence of OSA include neck size, and retroglossal space (indicative of mandibular retrusition and high palate). On a cellular level, leptin, a 167-amino acid hormone derived from adipocytes, having its effect via specific binding receptors in the brain and peripheral tissues, has been positively correlated with the amount of body fat. Binding of leptin activates signal transduction pathways, e.g. Janus kinase signal transducer, an activator of transcription-3 (JAK-STAT 3), instrumental in regulation of energy homeostasis, and phosphatidylinositol 3-kinase (PI3K), important for regulation of food intake and glucose homeostasis. Leptin levels have far reaching ramifications including cardiovascular disease. When controlling for BMI, leptin levels have been found to be higher in individuals with OSA compared to those without suggesting a level of leptin resistance in OSA compared to obesity alone.

1.4.3 EVALUATION OF SLEEP APNOEA

The assessment of sleepiness is central to the diagnosis of OSA, both in terms of establishing symptom severity and observing an effect of therapeutic interventions. Due to cost-ineffectiveness and time taken to perform multiple sleep latency test, it is not widely available. Since 1991, the Epworth Sleepiness Scale (ESS) has been used to assess and measure daytime sleepiness. The ESS asks respondents to determine how likely they are to sleep in eight daily situations, using a 4 point scale. Sleep propensity is the main factor measured by the ESS,
and using Cronbach’s alpha statistic, a value to 0.88 for this attribute suggests a high level of consistency and minimal redundancy. There are several omissions from the questionnaire such as whether the respondent has had a car accident in a moving vehicle, as opposed to a vehicle stationary at traffic lights. It also does not ask for evaluation of sleepiness in the work place. Furthermore, it does not include estimations of caffeine intake, changes in work and memory performance, planned naps or overall respondent energy levels. However, there is modest correlation with related domains of well-validated quality life assessment scores such as the SF-36 (the most strongly correlated $r=-0.47$, $p <0.001$), the Physical Component Score and the Mental Component Score. The Sleep Heart Health Study indicated that the ESS increased significantly with the AHI, while others have shown a significant correlation between ESS score and minimum oxygenation during sleep, not all studies have confirmed these relationships.

The Berlin questionnaire (BQ) has been used to help identify patients at greater risk for OSA, performing with high sensitivity and moderate specificity (0.97;054 respectively). Not all studies have demonstrated this however, with some demonstrating a poor specificity and sensitivity for the BQ in a sleep clinic population. The BQ includes five items on snoring, three items on daytime somnolence and one item for a history of hypertension. It also includes information about age, gender, height, weight and neck size. The overall score is dependent on the three categories. High risk patients are those with positive scores on two or more of the three categories.
The first continuous overnight EEG sleep recordings in humans were published in 1937, and the combination of breathing and monitoring of brain activity to identify pathological conditions developed in the mid-twentieth century.\textsuperscript{500} It is generally agreed that measurements should include 1 EEG derivation, 2 electrooculogram derivations, and 1 electromyogram. Other measurements include airflow, oxygen saturation, chest and abdominal muscle use, body position and leg movements, heart rate and ECG. The standard for staging is based on the Rechtschaffen and Kales and the American Academy of Sleep Medicine manual for sleep scoring.\textsuperscript{600,601} Due to the expense and difficulty with availability in major institutions, portable devices have been developed and validated.\textsuperscript{602,603} There are several types of devices, which can have from 1-7 leads for recording only oximetry, through to all the required measurements of a sleep laboratory.\textsuperscript{604,605} Choosing appropriate populations are important when considering using portable devices and it is essential to exclude patients with potential confounders including

1.4.4 PATHOPHYSIOLOGY OF OBSTRUCTIVE SLEEP APNOEA

1.4.4.1 NORMAL SLEEP

The process of sleep, the “reversible behavioural state of perception disengagement from and unresponsiveness to the environment”\textsuperscript{606} results in a number of complex behavioural and physiological processes, that can broadly be considered in 2 states based on electroencephalographic (EEG), electrooculographic (EOG) and electromyographic (EMG) characteristics: Non-rapid eye movement
(NREM) and rapid eye-movement (REM). NREM, consists of 4 stages and is associated with slow rolling eye movements, a synchronized EEG, and mildly reduced EMG tone. By comparison, REM sleep is associated with a desynchronized EEG similar to wakefulness, but characterised by theta or saw tooth waves, rapid eye movements and moderately-severely reduced muscle tone. This is the stage associated with an active mental state, dreaming and multiple arousals. With cycling through the stages of sleep, the body undergoes somatic and autonomic changes which, broadly speaking, include an increase in parasympathetic tone and a decrease in sympathetic tone in NREM sleep, with a further increase of parasympathetic tone and decrease of sympathetic tone in REM sleep, punctuated by intermittent increases in sympathetic activity. The autonomic restfulness of NREM sleep, where adults spend ~85% of their sleep time, is marked by cardiovascular calm - heart rate, blood pressure, stroke volume and cardiac output as well as systemic vascular resistance all decrease. Atrial and ventricular ERP effective refractory period lengthen during the night and sleep suppression of ventricular arrhythmias is observed. Furthermore, as a result of decreased tonic drive and increased upper airway resistance, there is a significant change in the parameters of breathing with progression from wakefulness through NREM sleep stages with an increase in the PaCO$_2$ and a decrease in PaO$_2$ incrementally from stage 1 to 4.

1.4.4.2 CONTRIBUTORS TO OBSTRUCTIVE SLEEP APNOEAS

OSA is defined by the interruption of airflow in the face of ongoing respiratory effort, whereas in contrast, the hallmark of central sleep
apnoea (CSA) (of which there are various forms such as Cheyne-Stokes respiration) is cessation of airflow without respiratory effort. There is well described mechanistic and clinical overlap between the two conditions.617

The main contributors to OSA pathophysiology are the anatomic features and functional state of the pharyngeal muscular airway, and the central neurochemical control of breathing stability and loss of neuromuscular control of the upper airway during sleep, leading to pharyngeal collapse. The pathophysiological consequences of the repeated interruption to breathing include i) hypoxia and hypercapnia, ii) generation of negative intrathoracic pressure and iii) multiple arousals from sleep.470,618

1.4.4.2.1 ANATOMICAL CONSIDERATIONS

In humans, compared to other mammals, the upper airway hyoid bone is not attached to the styloid processes of the skull. Rather, the human pharynx has rigid support only at its upper and lower ends, where it is attached to bone and cartilage. Consequently, pharyngeal cross-sectional area is highly dependent upon luminal pressure.619,620

In patients with OSA, the primary abnormality is an anatomically small airway.570 Imaging studies have demonstrated that the cross-sectional area of the upper airway during wakefulness is reduced in patients with OSA compared to those without. The sites of occlusion and narrowings of the pharyngeal airways are also multiple and varied among OSA patients, with the retropalatal region of the oropharynx being the most common site of collapse.621,622 This may result from anatomical structural abnormalities such as tonsillar and adenoid
hypertrophy, micrognathia, retrognathia, macroglossia as well as obesity and craniofacial abnormalities. The resultant smaller bony enclosures have been shown to be associated with pharyngeal collapsibility. This occurs posterior to the tongue, uvula and soft palate, as it is this segment of the airway from the posterior nasal septum to the epiglottis that has minimal supporting rigidity from bony structures, rendering it reliant on muscle activity for maintenance of patency. Even in individuals without obvious anatomical predisposition, visualisation of the airway with radiological imaging and endoscopic techniques has revealed a narrower upper airway. Independent of the presence of fatty deposits or fat pad thickness patients with OSA have been shown to have increased thickness of the lateral pharyngeal walls, resulting in a reduction of the lateral diameter of the airway.

In addition to the size of the airway, another important factor is airway shape, with several studies reporting a more oval shape in patients with OSA leading to a reduction in muscular control of the airway, resulting in less dilatation of the pharynx. Any insult to the upper airway that enlarges the soft tissues, such as accumulation of small amounts of fluid or vascular engorgement, including blood redistribution on recumbency, can lead to airway compromise and has been associated with OSA. Surface tension of the mucosa has also been shown to be important in airway patency with a higher upper airway wall surface tension reported in OSA sufferers. This may be improved with surfactant therapy by up to 30%. The route of breathing may alter these surface forces—nasal breathing may
influence salivary flow and result in a reduction of surface tension forces, whilst oral breathing may increase them.\(^{636}\)

1.4.4.2.2 MECHANICAL CONSIDERATIONS

Collapsibility of the airway can be quantified by assessment of the pharyngeal critical closing pressure (P\(_{\text{crit}}\)), the airway pressure, below which airway collapse occurs.\(^{637}\) In the paralysed pharynx of OSA subjects undergoing general anaesthesia, Isono et al. demonstrated ‘collapsibility’ at the site of primary closure at atmospheric pressure (the velopharynx), finding that the velopharyngeal closing pressure was the only variable highly correlated with the oxygen desaturation index, and that this was the principal correlate of the frequency of nocturnal desaturations.\(^{620}\) By comparison, normal patients did not lose airway patency at atmospheric pressure. This collapsibility of the airway is due to many factors including the cross-sectional area of the upper-airway, adipose tissue deposition around the airway, airway length, lateral pharyngeal wall thickness and tongue volume.\(^{572}\)

In the normal individual, during passive conditions (i.e. where the muscle is completely inhibited) the airway remains patent and requires about -5 cm H\(_2\)O for it to collapse.\(^{571,574,638,639}\) Patency of the airway is maintained because the extraluminal tissues do not provide sufficiently positive pressure to overcome the elasticity of the airway. However, in OSA individuals, the quantity of extraluminal tissue relative to the size of the airway is such that a sufficiently great positive pressure is applied to the airway, which leads to its partial or complete collapse.\(^{572}\) Obesity, a major risk factor for OSA, has been associated with increased neck circumference and peri-\(^{568,572}\) and para-\(^{575}\) pharyngeal fat, which has
been correlated with increased OSA severity. In addition to the quantity in extraluminal tissue found in obese individuals, obesity may also lead to collapse of the pharyngeal airway via reductions in tracheal traction on the pharyngeal segment, resulting from a reduction in functional residual capacity owing to reductions in lung volumes. This is further exemplified by observing the effect of continuous negative airways pressure on upper airway collapsibility, via its effect on decreasing lung volume. The opposite effect, i.e. an improvement in airway collapsibility, can be achieved with increasing lung volume.

Gender differences in Pcrit exist and may be related to anatomical differences in pharyngeal airway length and pharyngeal soft tissue mass, as well as distribution of extrapharyngeal fat (i.e. in the upper body and trunk). Given the above, treatment for OSA relies on increasing the Pcrit with CPAP (i.e. widening the differential pressure between upstream pressure and Pcrit and preventing it from falling below Pcrit and collapsing the airway). A reduction in the Pcrit has also been demonstrated with weight loss or alteration of craniofacial anatomy, increasing lung volume or neuromuscular augmentation.

1.4.4.2.3 NEUROMUSCULAR CONSIDERATIONS

Important in counteracting the collapsing force of airway pressure are three groups of inspiratory dilator phasic upper-airway muscles—those of the hyoid bone (geniohyoid and sternohyoid), the tongue (genioglossus) and the palate (tensor palatini, levator palatini). These muscles all have controlling neurons situated in the brainstem.
Remmers et al. highlighted this important component of the airway by demonstrating the reduction in genioglossal electromyogram activity at the onset of apnoea, with reversal on restoration of airway lumen patency. Furthermore, Mezzanotte et al. demonstrated significantly greater basal genioglossal activity compared to normals without OSA (40% versus 12%) speculating that the neuromuscular compensation present during wakefulness was diminished during sleep, thus contributing to airway collapse. Further studies by the same group demonstrated larger decrements in muscle EMG activity in OSA patients than in controls, representing loss of neuromuscular compensation during sleep.

Additional to central mechanisms, local reflexes also contribute to muscular upper airway tone. Pillar et al. demonstrated that these respond to negative pharyngeal pressure in their studies on individuals following delivery of topical airway anaesthesia, which resulted in decrements in phasic genioglossus EMG activity, increased pharyngeal resistance and a reduction in the relationship between negative epiglottic pressure and genioglossus activity. Further demonstrative studies were conducted by Malhotra et al. on patients undergoing tracheotomy; there was significant reduction in tonic genioglossus activation during stomal breathing compared to nasal breathing, suggesting that local upper airway respiratory stimuli were important in activation of the pharyngeal dilator muscles. The complexity of this reflex arc was demonstrated in studies indicating both excitatory and inhibitory stimulatory components were differentially affected by sleep state in OSA patients. During wakefulness, there was compensation for an
anatomically narrow upper airway by a negative-pressure reflex, which caused genioglossus muscle activity leading to upper airway dilatation. During sleep, there was diminution of this (i.e. genioglossus EMG suppression amplitude) during NREM, with further during REM.\textsuperscript{650} Hence, more pronounced reflex inhibition of muscular activity, rather than loss of excitation, may be important during sleep, and this may be greater for OSA patients than for healthy individuals.\textsuperscript{650,651}

There is suggestion from several studies that upper airway repetitive collapsing causes local trauma that results in injury to neuronal fibres and subsequent sensorimotor dysfunction, which may partially reverse with CPAP.\textsuperscript{652} Palatopharyngeus and muscularis uvulae muscle biopsy studies by Edstrom et al. and others have shown “neurogenic lesions”, characterised by angulated atrophic fibres, twin or multiple peak distribution of the fibre size spectra and an abnormal distribution of fibre types\textsuperscript{653}. These neuromyopathic processes and their importance require further investigation.

In summary, a combination of two critical determinants accounts for an individual's propensity to upper-airway collapse during sleep - the predisposing anatomically narrow airway, and, the level of activity of the pharyngeal dilator muscles.

1.4.4.2.4 VENTILATORY CONTROL STABILITY

Ventilatory control stability is important as the patient with OSA cycles between sleep with obstructive breathing events and wakefulness, and has been described using the engineering concept of loop gain.\textsuperscript{654,655} In this context, loop gain refers to the control of the respiratory system and how it responds to the repetitive arousals that
cause changes to the general breathing pattern of sleep. There are two main components known as 1) controller gain and 2) plant gain. The chemoresponsiveness of the system is the controller gain (i.e. how ventilation responds to hypoxaemia and hypercapnia). Plant gain refers to the ability of the system to excrete CO$_2$ (i.e. how efficient a level of ventilation is at excreting CO$_2$). The instability in the system is a result of the physical separation of the sensors and effectors in the loop.$^{656}$

Hypercapnia and hypoxia are detected by peripheral and central chemoreceptors, which result in an increasing central drive to the upper airway, and hence decreasing pharyngeal collapsibility.$^{657,658}$ The PaCO$_2$ level below which apnoea occurs is known as the apneic threshold. During sleep this is unmasked, whereby apnoea is initiated by small transient reduction in PaCO$_2$. Brisk breathing, resulting from an arousal when the PaCO$_2$ is at this threshold, can lead to apnoea on resolution of the arousal and return to sleep.$^{659}$ In comparison, during wakefulness there is a transient increase in central respiratory motor output, which remains for a short time after cessation of hyperventilation, preventing apnoea from occurring and allowing ventilation to return to eupnoeic levels.$^{660,661}$ Arousals, along with reductions in upper airway resistance and increasing chemoreceptor drive from the preceding ventilatory undershoot, are very effective at causing these ventilatory overshoots and hypercapnia resulting in central or obstructive apnoeas (i.e. there is increased plant gain).$^{662-664}$ Studies using proportional assist ventilation techniques have confirmed patients with OSA demonstrate elevated loop gain and suggest that ventilatory instability is an important mechanism in the pathogenesis of the disorder.$^{665,666}$
1.4.4.2.5 AROUSALS

In order to prevent asphyxia, termination of apnoea requires a transient arousal, allowing activation of the upper-airway muscles and re-establishment of airway patency. Several mechanisms of arousal have been described and include stimulation of central and peripheral chemoreceptors by the hypercapnia and hypoxia, afferent inputs arising from the airway, lung and chest wall receptors leading to increasing ventilatory effort, and apnoea-activated stimulation of the reticular activating system by respiratory neurons. 667-669

1.4.4.3 PATHOPHYSIOLOGY DUE TO OSA

There are several consequences of OSA that may contribute to the development of AF. The potential role for inflammation, endothelial dysfunction and oxidative stress have been discussed in previous sections.

1.4.4.3.1 CARDIAC STRUCTURAL CHANGES

As a result of the changes caused by OSA, several cardiac structural abnormalities have been documented. In one study examining the effects of severe OSA, only 8% of the subjects had normal findings on echocardiography. 670 Left ventricular hypertrophy (LVH) was the most common finding (88%), left atrial enlargement the second most common (64%), followed by right atrial enlargement in 48% and right ventricular hypertrophy in 16% of the subjects. 670 LV function was normal in this study. Other studies have shown a high prevalence of OSA in the heart failure population, as well as patients with heart failure and preserved LV function. 671
Whilst hypertension is a contributing factor to LVH, it is present in roughly half of OSA patients with myocardial thickening, suggesting that increased transmural pressure, hypoxaemia and increased sympathetic neural activity may also be contributing factors.\textsuperscript{670,672} Other studies have documented an increase in LV mass and LV mass index associated with OSA.\textsuperscript{673} However, these findings have not been replicated in many other studies, where LV mass increase was thought to be predominantly due to coexisting findings of obesity, hypertension and age.\textsuperscript{674,675} After controlling for other factors, the importance of the hypertension was demonstrated by its close association with OSA and the presence of LV hypertrophy.\textsuperscript{676} Many studies have also demonstrated the beneficial effects of CPAP on blood pressure control and LVH.\textsuperscript{670,677}

Obstructive apnoeas cause a negative intrathoracic pressure during inspiration, leading to an increase in the LV transmural pressure and thus afterload, culminating in LV hypertrophy independent of the effect of hypertension.\textsuperscript{673} The augmented transmural pressure across the LV wall also causes an increase in the myocardial oxygen demand and a reduction in coronary blood flow.\textsuperscript{673,678-680} Together with the hypoxia resultant from the obstruction, there is myocardial ischaemia, impairment of contraction and diastolic relaxation, and this is linked to the degree of OSA.\textsuperscript{681}

Echocardiographic studies have shown variable results for RV hypertrophy changes in OSA, ranging from 0-71\% depending on whether patients had normal or abnormal daytime arterial blood gases.\textsuperscript{672,673,682,683} Noda et al. related these changes to the development of pulmonary hypertension in these patients.\textsuperscript{672} However, Sanner et al.,
who found an association between the degree of OSA and pulmonary hypertension, did not find a relationship between pulmonary hypertension and RV ejection fraction.\textsuperscript{684} Earlier studies by Fletcher et al. demonstrated improvements in both pulmonary haemodynamics and RV ejection fraction with treatment for OSA.\textsuperscript{685} Subsequent to the hypoxia induced vasoconstriction in the pulmonary bed and the increase in preload caused by increase in venous return, RV distension and septal displacement leading to impairment of LV filling was observed.\textsuperscript{686}

With the development of LV diastolic dysfunction, there is impairment of LA emptying, as the myocardium stretches to maintain adequate ventricular filling. This has been shown to have relevance and implications for atrial fibrillation and cardiovascular mortality.\textsuperscript{687,688} LA size has been found to be enlarged in individuals with OSA, independent of other important risk factors such as obesity.\textsuperscript{689} Using more advanced techniques, Oliveira et al. demonstrated important changes in LA functional measurements, showing a significant increase in LA volume and LA active systolic function with impairment of LV diastolic function, independent of hypertension and obesity.\textsuperscript{690} Interestingly, a further study by the same group demonstrated a beneficial effect of CPAP on LV diastolic dysfunction with an improvement in passive LA emptying and a reduction in active emptying—the overall effect being no significant change in LA total emptying fraction and no change in absolute LA volumes.\textsuperscript{691} Abrupt changes to LA volume and LV systolic performance associated with an increase in LV afterload have been noted when the Mueller manoeuvre is performed.\textsuperscript{692}
Additional to this, hypoxaemia may impair ventricular relaxation as a result of the hypoxic effects on intracellular calcium transport, reducing the removal of calcium from the cytosol. In a recent study, systolic blood pressure and mean nocturnal oxygen saturation were shown to be independently associated with the presence of left ventricular hypertrophy, with logistic regression modelling demonstrating age (OR 3.29) and mean nocturnal oxygen desaturation <92% (OR 2.76) to be associated with left ventricular diastolic dysfunction.

A recent study has documented the effects of CPAP treatment on cardiac structure in OSA patients. Using both transthoracic echocardiography and cardiac MRI, this study found that there was positive cardiac remodeling over the long-term, characterised by a reduction in RA and RV chamber dimensions and volume, improvement in LA diastolic function with a reduction in chamber volumes and LV filling pressures, and ultimately, regression of LV mass. These changes were noted within the first 3 to 6 months of CPAP treatment with continued improvement in the 12 months after institution of CPAP therapy.

1.4.4.3.2 AUTONOMIC NERVOUS SYSTEM CHANGES

The effects of both arms of the nervous system on the atrial myocardium and the implications for AF have been discussed in previous sections. In a normal individual without OSA, during NREM sleep (which compromises about 85% of adult sleep), there is a progressive decline in sympathetic output with an increase in parasympathetic tone. This results in a state of haemodynamic calm and cardiac electrical stability, demonstrated by the reduction in ventricular
premature beats in sleeping subjects.\textsuperscript{486,695,696} This state of quiescence is interrupted by recurrent adverse respiratory events in patients with OSA, resulting in recurrent hypoxaemia and changes in autonomic tone. There are several possible mechanisms responsible for these changes in autonomic tone and consequently the development of arrhythmias.

1.4.4.3.2.1 CENTRAL PROCESSES

Experiments in dogs under controlled circumstances where the influence of phasic chest wall movements and thoracic pressure swings were removed, have examined respiratory sinus arrhythmia using measures of heart rate and averaged phrenic neurograms.\textsuperscript{697} They found that respiratory sinus arrhythmia was centrally mediated and directly related to respiratory drive rather than other proposed mechanisms such as pulmonary stretch receptors, baroreceptors, mechanoreceptors, chemoreceptors or any other phasic afferent information.\textsuperscript{697} Similar conclusions on the influence of central mechanisms have been drawn from studies on the central modulation of sympathoexcitation in rats.\textsuperscript{698} In humans, similar observations have come from lung transplant recipients.\textsuperscript{699} In this setting, there is absence of intrathoracic or intravascular receptors and lung-inflation stimulated vagal afferents. Measurements of skeletal muscle sympathetic nerve activity (MSNA), in the absence of these mechanisms, have pointed to centrally mediated pathways. The ‘diving reflex’ - the pattern of respiratory, cardiac and vascular responses triggered by breath-hold in diving - has been shown to be present in breathing animals in response to conserving oxygen during submersion.\textsuperscript{700} This response to apnoea consists of vasoconstriction due to sympathetic activity, initial hypertension and a
vagally induced bradycardia, with subsequent reduction in cardiac output. This is another centrally mediated medullary based reflex. 700

1.4.4.3.2.2 LUNG REFLEXES

Lung inflation via pulmonary stretch receptors and the pulmonary vagal nerve causes a reduction in sympathetic neural activity. 701 The reduction in stretch caused by apnoeas in OSA patients may result in withdrawal of this sympathoinhibitory influence and a greater sympathetic outflow, particularly in the presence of hypoxia. 702 Additionally, in the presence of hypoxia (which acts via peripheral chemoreceptors and hypercapnia modulating ventilation), and sympathetic output via central chemoreceptor stimulation, the effect on sympathetic outflow is synergistic. 702 At high lung volumes, the Hering-Breuer reflex is initiated, and allows for reflex inhibition of inspiratory neurons in the brainstem. This is also triggered by pulmonary stretch receptors, which may affect sympathetic activity indirectly. 703

1.4.4.3.2.3 CARDIAC REFLEXES

Cardiac reflexes, the afferent receptors located in the RA and LA, and, the caval and pulmonary veins, respond to central venous pressure. This causes attenuation of vagal efferent activity and a reflex increase in the heart rate. 704,705 Studies on heart failure patients have suggested the presence of sympathoexcitatory reflexes triggered by increased filling pressure, which may contribute to sympathetic outflow in this subgroup of patients with OSA. 706
1.4.4.3.2.4 INTRATHORACIC PRESSURE

The intrathoracic pressure changes, such as that simulated by the Mueller manoeuvre, which are present in OSA, have been shown to have a significant effect on cardiac haemodynamics, by decreasing LA volume and increasing LV end-systolic volume, culminating in a reduction in stroke volume. During the manoeuvre, as demonstrated by Somers et al., sympathetic activity is initially inhibited. This is perhaps due to the effect of stretch on the aortic baroreceptors opposing hypotensive stimulation of carotid baroreceptors, the intraventricular negative pressure and atrial transmural gradients. As the manoeuvre is maintained, there is an increase in sympathetic activation due to adaptation of these mechanisms, hypoxia and hypercapnia (see later). On release, there is sympathetic inhibition caused by the sympathoinhibitory effects of lung inflation, normalisation of oxygen and carbon dioxide levels, and a surge in blood pressure due to baroreceptor mediated pathways. On the other hand, end expiratory apnoea (i.e. without the Mueller manoeuvre) results in more sustained elevation in sympathetic activity. Adding further complexity, in OSA, the arterial baroreflex is downregulated, however, baroreflex sensitivity is augmented with institution of CPAP. This may contribute to the chronically increased sympathetic tone seen in patients with OSA.

1.4.4.3.2.5 HYPOXIA AND HYPERCAPNIA

Carotid chemoreceptor sympathetic outflow, along with increases in blood pressure and ventilation are augmented in response to the acute and chronic intermittent hypoxia present in patients with OSA. Experimental acute hyperoxia (100% oxygen) decreases
this hypersensitivity, resulting in a reduction in MSNA and blood pressure in OSA subjects.\textsuperscript{713} Animal models demonstrate that in chronic exposure to intermittent hypoxia, there is a long-lasting increase in baseline carotid body sensory activity, known as ‘long-term facilitation’. This is linked to reactive oxygen species (generated by NADPH oxidase activity), which may be reversed with normoxia.\textsuperscript{714,715} This may partially explain the positive effects of CPAP on sympathetic outflow.\textsuperscript{470} In addition, chronically elevated catecholamine levels seen with OSA may be augmented by hypoxia induced catecholamine secretion from the adrenal medulla.\textsuperscript{716}

Hypercapnia has been shown to be a potent stimulator to ventilation and sympathetic activity via the central chemoreceptors, more so than hypoxia, and even more so in the presence of hypoxia.\textsuperscript{702} Moreover, there appears to be inhibition of pulmonary afferents, resulting in a reduction of the inhibitory effect and an increase in net sympathetic tone despite tachypnoea.\textsuperscript{702} Evidence from heart failure patients points towards not only hypercapnic stimulation of SNS, but also increased central chemoreflex sensitivity.\textsuperscript{717}

1.4.5 ADVERSE CLINICAL CONSEQUENCES OF OBSTRUCTIVE SLEEP APNOEA

1.4.5.1 HYPERTENSION

The strong relationship between OSA and hypertension has been demonstrated in many cross-sectional and longitudinal studies. Cross-sectional analysis of the 6132 subjects in the Sleep Heart Health Study demonstrated increasing blood pressure (BP) with higher degrees of
OSA, and hence greater prevalence of hypertension.\textsuperscript{718} This relationship was observed after adjusting for demographic and anthropometric variables, and was noted across age groups, gender and ethnic groups in both overweight and normal-weight individuals. Peppard et al. examined 709 patients from the Wisconsin Sleep Cohort at baseline and after 4 years, adjusting for baseline hypertension, measures of obesity, age, gender, alcohol and cigarette usage, they found an independent dose-response relationship between the presence of OSA and hypertension with an OR of up to 2.89 with AHI \textgreater{} 15.\textsuperscript{719} This data was further explored in a 328 subject sample derived from the same group of patients. Over 7.2 years, and adjusting for covariates, there was a dose-response relationship for the development of systolic non-dipping BP, with worsening AHI.\textsuperscript{720}

From the perspective of drug-resistant hypertension, one study with patients on high doses of at least three antihypertensives, showed a prevalence of OSA of 83\% amongst the 41 patients studied.\textsuperscript{721} In a larger study, of 1,485 patients with OSA, 393 reported taking medication for hypertension, of which 74 patients were ineffectively controlled.\textsuperscript{722} In comparing the effectively and ineffectively controlled groups, after accounting for covariates such as age, gender and BMI, the ineffectively treated patients had higher AHI, despite having similar nocturnal oxygenation parameters.

CPAP therapy has been found to have a beneficial effect on the treatment of hypertension in OSA patients. This has been demonstrated in several trials such as that by Wilcox et al.,\textsuperscript{677} and, subsequently, a number of meta-analyses.\textsuperscript{723-725} In the aforementioned study using 24
hour ambulatory BP, measured before and after 8 weeks of nasal CPAP therapy in 19 men, both systolic blood pressure (SBP) and diastolic blood pressure (DBP) fell significantly (systolic during the night and day, diastolic only during the day) in both normotensive and hypertensive individuals, independent of changes in body weight or alcohol consumption. The meta-analysis by Alajmi et al. suggested that the greatest benefit was obtained in patients with more elevated AHI, and that individuals with less severe OSA may only derive modest benefit at best. This was again borne out in the review by Haentjens et al. who showed that the net decrease due to CPAP was 1.77 mmHg for SBP and 1.79 mmHg for DBP, and that 24-hour mean BP would decrease by 0.89 mmHg per 10-point increase in baseline AHI and by 1.39 mmHg for each 1 hour increase in effective nightly dose. This suggested again that more severe OSA sufferers would derive most benefit and that the duration of effective treatment was clearly important.

Overall, there is an association with OSA and hypertension, AHI is an independent risk factor for hypertension, and treatment of OSA with CPAP lowers the BP, albeit the effect is likely to be more noticeable in drug-resistant hypertension in patients with severe OSA. However, there are several confounding factors that are not accounted for in these trials, including physical activity and dietary habits. Whether this can be translated into a decreased risk of cardiovascular events independent of modification of other known risk factors is currently unknown, but may be addressed by the results of current, and future trials.

Mechanisms responsible for the association are several. Cycling of hypoxia and hypercapnia can stimulate the SNS, resulting in
peripheral vasoconstriction and hence an increase in systemic BP.\textsuperscript{728,729} This augments the afterload increase brought about by generation of the negative intrathoracic pressure caused by airway occlusion. Repeated arousals triggering termination of apnoea results in an increase in sympathetic tone and a reduction in vagal output, which consequently causes a sudden surge in BP.\textsuperscript{485} The effects on BP are not limited to nocturnal surges and elevation but are sustained during the daytime, indicative of a carryover effect and ongoing pathology during wakefulness.\textsuperscript{730,731} Other consequences of OSA and intermittent hypoxia previously discussed include induction of oxygen free radicals, activation of inflammatory pathways and impairment of endothelial function, which may all lead to systemic hypertension independent of activation of the SNS.\textsuperscript{354,370,438,732,733}

\subsection{1.4.5.2 INSULIN RESISTANCE, DIABETES AND DYSLIPIDAEMIA}

OSA is strongly associated with central obesity. In some studies, this has been found to be a better predictor of OSA than neck circumference.\textsuperscript{734} The relationship between central obesity and insulin resistance and diabetes mellitus is well described.\textsuperscript{735} Linking these two important relationships, the term “Syndrome Z”, coined by Wilcox et al., has been used to describe the association between typical features of Syndrome X (i.e. central obesity, hypertension, diabetes, dyslipidaemia) with the addition of OSA.\textsuperscript{18} Many studies have demonstrated the presence of increased plasma insulin levels in patients with documented OSA. These studies have shown a relationship between insulin resistance and the severity of nocturnal hypoxaemia,\textsuperscript{736-740} the association of OSA with type II diabetes mellitus, independent of OSA’s
relationship with obesity. Furthermore, CPAP therapy has been shown to improve insulin resistance in the short and long term. Pathogenic mechanisms involved in the genesis of this relationship revolve around the intermittent hypoxia and sleep fragmentation central to the diagnosis of OSA, and the consequences of sympathetic activation, oxidative stress, inflammatory pathway activation, hypothalamic-pituitary-adrenal axis dysregulation and the subsequent release of corticosteroids. Intermittent hypoxia also leads to changes in adipokines secreted from adipose tissue, such as leptin, which acts to inhibit insulin secretion and affects glucose uptake. This has been shown to be reversed with institution of CPAP.

Cross-sectional studies have revealed mixed results with regard to dyslipidaemia, with only some demonstrating a relationship between OSA and an increase in lipid levels. The reasons are difficult to determine, but most studies were not designed primarily to examine lipid profiles and did not take into account important confounders such as physical activity and diet. There are animal studies that do demonstrate an effect of intermittent hypoxia on lipid profiles with proportionality to the degree of induced hypoxia. The mechanisms appear to be dependent on hypoxia induced upregulation of hepatic lipid biosynthetic pathways, and free-fatty acid lipolysis and excretion from adipose tissue.

1.4.5.3 CORONARY ARTERY DISEASE

The most compelling evidence for the relationship between cardiovascular disease and OSA comes from the Sleep Heart Health Study, which reported an independent relationship between self-reported
OSA and heart failure (OR 2.38), cerebrovascular disease (OR 1.58) and coronary artery disease (OR 1.27), when comparing the upper and lower most quartiles of AHI. Over a 7 year period, the effective treatment of OSA with CPAP resulted in a reduction in the risk of developing cardiovascular disease after adjusting for covariates.\footnote{564} In a sample of patients without heart disease at baseline, over 7 years, coronary artery disease was observed in 16.2\% of 105 patients with OSA compared to 11\% of 203 snorers without OSA. Here, the presence of OSA at baseline was an independent predictor with an RR of 4.6.\footnote{768} This was again demonstrated in a prospective study on patients with cardiovascular risk factors and OSA. Those that were intolerant of CPAP had more cardiovascular events compared to those effectively treated with CPAP (31\% vs. 18\%, $p<0.05$).\footnote{769} In a study on 78 patients undergoing percutaneous coronary intervention for coronary stenosis, patients with OSA had more late lumen loss than those without, suggesting a greater degree of vessel remodeling in these patients.\footnote{770} Another study has demonstrated a greater incidence of revascularisations and cardiac mortality in patients with OSA being an independent predictor of these.\footnote{771} Again, treatment of OSA with CPAP has beneficial effects, with a reduction in cardiac death after percutaneous coronary intervention.\footnote{772}

Pathophysiological mechanisms important to the development and perpetuation of cardiovascular disease (discussed elsewhere in this thesis) include OSA’s association with obesity, diabetes, dyslipidaemia and hypertension, as well as the consequences of intermittent hypoxia/hypercapnia, including SNS activation, oxidative stress, inflammation and endothelial dysfunction.
1.4.5.4 STROKE

In a community study on 394 older subjects aged 70 to 100 years, there was a 2.5 fold increase in the risk of ischaemic stroke associated with severe OSA.\textsuperscript{773} In a cross-sectional analysis conducted as part of the Wisconsin Sleep Cohort, moderate to severe OSA was associated with a 3 times risk of a previous stroke, after adjusting for confounders such as age, gender, and BMI.\textsuperscript{774} To address the incidence of stroke, Redline et al. reported on 5422 participants with untreated OSA and without stroke from the Sleep Heart Health Study. Following for a median of 8.7 years, men in the highest quartile AHI had an adjusted HR of 2.86 for stroke. In the mild-moderate OSA groups, the risk of stroke rose by 6% for every 1-unit increase in AHI. By comparison, an increased risk of stroke was only recognized female participants with an AHI >25.\textsuperscript{775}

1.4.5.5 CARDIOVASCULAR MORTALITY

Mortality is increased in the presence of OSA, as demonstrated in observational cohort studies. In the Wisconsin Sleep Cohort, there was an increase in all-cause mortality when adjusted for covariates including age, gender, and BMI, with a HR for severe OSA compared to no OSA of 3.0, and of 3.8 when patients using CPAP were excluded from the analysis.\textsuperscript{776} Cardiovascular mortality alone conveyed a HR of 5.2.\textsuperscript{776} The Busselton Health Study from Western Australia demonstrated an all-cause mortality HR of 6.24 over 14 years when comparing moderate-severe OSA to non-OSA individuals. An AHI of 5-15 was not associated with an increased risk of dying.\textsuperscript{777} A much larger study, the Sleep Heart Health Study, enrolling over 600 participants with a follow-up of just over...
8 years, demonstrated a lower fully adjusted HR (but with smaller confidence intervals) for all-cause mortality - 1.46 (CI, 1.14-1.86). Milder OSA demonstrated a HR of 1.17 (CI, 0.97-1.42). Measures of intermittent hypoxaemia such as percentage of total sleep time with an oxyhaemoglobin saturation below 90%, was independently associated with all-cause mortality, but only in men below the age of 70.\textsuperscript{778} An observational study comparing the incidence of fatal and non-fatal cardiovascular events in OSA sufferers and healthy recruits demonstrated that untreated OSA had an increased OR for fatal (2.87) and non-fatal (3.17) cardiovascular events, and that CPAP treatment reduced that risk.\textsuperscript{779}

To date, there are no randomized trials to examine whether CPAP reduces all-cause mortality. However, a large trial (with a recruitment target of 5000 patients) is currently underway—SAVE (Sleep Apnoea Cardiovascular Endpoints Study).\textsuperscript{780} This study, an academic initiated and conducted, multinational, open, blinded endpoint, randomised controlled trial, is designed to address the role of CPAP in recruited patients with OSA and cardiovascular disease, analysing hard endpoints of myocardial infarction and stroke.

1.4.5.6 SUDDEN CARDIAC DEATH

In The Sleep Heart Health Study, increasing OSA quartile and severity of hypoxia were both significantly associated with increasing complex ventricular ectopy. Non-sustained ventricular tachycardia was experienced by 5% of those with severe OSA.\textsuperscript{781} Two important studies have demonstrated an increased risk of sudden cardiac death (SCD) with OSA. In one long term follow-up in 168 patients, death and new
cardiovascular disease combined, was more common in a group not effectively treated with CPAP (148% vs. 1.9%, p=0.009). More convincing work published by Gami et al. examined 112 death certificates in residents who had undergone polysomnography and died suddenly. They reported that for people with OSA, the relative risk of death from cardiovascular causes was 2.57 between the times of midnight and 6 a.m. and that the risk correlated directly with the AHI.

CPAP is likely to have beneficial effects, on the outcomes from death rates in cardiovascular diseases, although further studies are required to determine whether the risk of SCD is reduced per se. Potential mechanisms of risk reduction could include a reduction in ventricular arrhythmia mediated by improvement of nocturnal oxygen saturation, a reduction in sympathetic outflow and improved ventricular function, as demonstrated in the heart failure population.

1.4.5.7 ATRIAL FIBRILLATION

In 1983, Guilleminault et al. authored the first study of cardiac arrhythmias and OSA. Of the 400 patients studied, 48% had cardiac arrhythmias at night. They reported a prevalence of nocturnal paroxysms of AF of greater than 3%. Interestingly, in this study, tracheostomy was performed in 10 patients with severe OSA and atrial arrhythmia, after which there was no observed recurrence of AF/atrial flutter during the six month follow up.

From The Sleep Heart Health two groups were examined, 228 individuals with OSA defined as an RDI ≥30 and 338 subjects free of the disorder. Arrhythmia presence was increased in the individuals with OSA, including AF (4.8% vs 0.9%, p=0.003), non-sustained VT and
complex ventricular ectopy.\textsuperscript{784} When controlling for the presence or coronary artery disease, age, gender and body mass index, there was an OR of 4.02 for the association between AF and OSA.

In a Japanese community study, 1,763 men aged 40 to 74 years, who participated in the 2000 to 2004 annual cardiovascular risk survey, were recruited for a sleep study.\textsuperscript{785} Using a 3% oxygen desaturation index to define sleep disordered breathing, and after adjustment for covariates, the OR for AF was 2.47 for those with mild OSA (AHI 5-15), and 5.66 for those with moderate to severe disease (AHI ≥15).

In a prospective study, on 151 consecutive patients undergoing DC cardioversion for AF compared to 312 patients in a general cardiology practice, Gami et al. found the proportion of patients with OSA (diagnosed with the validated Berlin questionnaire) was significantly higher in the AF group than in the general cardiology group (49\% vs. 32\%). On adjusted logistic analysis, the OR for the association between AF and OSA was 2.19.\textsuperscript{786}

In another prospective study by Kanagala et al., 43 patients identified as having OSA and recurrence of AF (27 not receiving CPAP) and 79 randomly selected cardioversion patients (controls) were followed.\textsuperscript{787} Recurrence of AF 12 months post-cardioversion in those patients not receiving CPAP was 82\%, compared to the 42\% recurrence in the treated OSA group and the 53\% recurrence in the control patients. Furthermore, in the untreated OSA patients, the nocturnal fall in oxygen saturation was greater in those who had recurrence of AF.

More recently, an important 20 year prospective study from the Mayo Clinic examined 3,542 Olmstead country adults with no known
history of past or present AF, who were referred for an initial diagnostic polysomnogram.\textsuperscript{21} Over a mean follow-up of 4.7 years, new onset, electrogram documented AF was found in 133 individuals, representing a cumulative probability of 14\%. Important univariate predictors of AF were BMI and the presence of any OSA (AHI $\geq$ 5; HR 2.18), as well as several measures OSA severity. On multivariate analysis, independent predictors were age, male gender, coronary artery disease, BMI (per 1 kg/m\textsuperscript{2}, HR 1.07) and a decrease in nocturnal oxygen saturation (per 0.5 U log change, HR 3.29). Heart failure was only found to be an independent predictor in subjects above the age of 65.

Contributing to our knowledge of the temporal relationship between respiratory events and the nocturnal presence of AF, Monahan et al. examined 2816 polysomnograms from the Sleep Heart Health Study.\textsuperscript{788} They found that the odds of an arrhythmia occurring after a respiratory disturbance were 17.5 times the odds of occurrence after normal breathing. In their study, there was no significant effect of hypoxia (oxygen saturation $\leq$ 92\%) or arousals defined by electroencephalogram criteria, and they concluded that neither the severity of the breathing disorder or the individual respiratory events needed to be extreme to result in an increase in the chance of an arrhythmia.

Some studies have now been published reporting the beneficial effects of OSA treatment. Guilleminault et al. documented that tracheostomy in 50 subjects with severe OSA and known atrial arrhythmia resulted in absence of these atrial rhythm disturbances in the ensuing 6 months of follow-up.\textsuperscript{19} In an observational study, Kanagala et
al. explored the recurrence of AF after cardioversion in the presence of OSA.\textsuperscript{787} They reported that patients with OSA had a higher recurrence of AF after successful cardioversion than patients without a sleeping disorder (82\% vs. 53\%). Moreover, patients with CPAP treated OSA had less chance of recurrent AF than untreated patients (42\% vs. 82\%). In patients with untreated OSA, there was a relationship between recurrence and the degree of oxygen desaturation. Stevenson et al. further explored the relationship between SDB and AF finding a greater prevalence and worsening degree SDB in AF patients compared to an age-matched non-AF population.\textsuperscript{789} Furthermore, in the patients with high frequency AF defined as greater than 6 episodes in the past year, there was a higher prevalence and severity of SDB when compared to low-frequency AF.

The effects of OSA on failure of AF interventional strategies have been demonstrated in several studies examining the interaction of OSA with AF ablation. In a report by Jongnarangsint et al 324 AF patients with paroxysmal or permanent disease, retrospectively, 10\% of the study population had a diagnosis of OSA prior to their ablation.\textsuperscript{790} Despite small number, multivariate analysis revealed OSA was the strongest predictor of recurrent AF after catheter ablation, with an OR of 3.04, independent of BMI. Other investigators, such as Chilukuri et al. have identified high risk Berlin questionnaire to be an independent predictor of failure after catheter ablation for AF, with an OR of 4.53. These studies and others have been examined together in an meta-analysis published during the time this thesis was being prepared, included the two above studies and 4 others.\textsuperscript{791} It concluded that OSA diagnosed on
polysomnography was considered a strong predictor of AF recurrence (RR 1.40), but Berline questionnaire was not useful. There was a 25% greater risk of AF recurrence after catheter ablation in patients with OSA.

### 1.4.6 ASSOCIATION OF AF AND CENTRAL SLEEP APNOEA

Often, during polysomnography for the evaluation of OSA, episodes of CSA are noted, although they are likely to be of little clinical significance.\(^7\) The presence of CSA on commencement of CPAP has been seen in patients with various disorders, including severe OSA, systolic heart failure, neuromuscular disorders and in those on opioids.\(^7\) In the presence of OSA, either diagnosed during diagnostic testing or developing after initiation of CPAP for OSA, together, they are known as “complex sleep apnoea”, with a prevalence of 5-6%.\(^7\) This thesis focuses on OSA, however as discussed below, CSA has been associated with AF and the two conditions may co-exist. Hence, a brief mention of CSA is warranted.

CSA is a “central” breathing disorder with the temporary failure of pontomedullary rhythm generation. Unlike OSA, in CSA, there is the absence of thoracic movement during apnoea as a result of the absence of brainstem neural output.\(^6\) The main mechanisms involved are related to a reduction in the drive to breath, with unmasking of the PCO\(_2\)-sensitive apnoeic threshold.\(^7\) CSA may be physiological e.g. with sleep onset and sometimes during REM sleep. Other causative conditions include systolic heart failure, post-stroke, various congenital and brainstem disorders, interruption of neural pathways, endocrine disorders such as acromegaly and hypothyroidism, and, in their absence, the disorder may be idiopathic (which is rare).\(^7\)
In heart failure, studies have demonstrated that 40-80% of patients have an AHI $\geq 15$ including central and obstructive apnoeas.\textsuperscript{797-799} A particular pattern of respiration is observed as a result of the long circulation time seen in heart failure, which causes “periodic breathing”.\textsuperscript{800} Mechanisms important to this disorder additional to the long circulation times include a lack of PCO$_2$ increase with sleep onset,\textsuperscript{801} increased CO$_2$ chemosensitivity below eupnoea,\textsuperscript{802,803} and reduction in PCO$_2$ reserve.\textsuperscript{792,804}

In 1999, a study on 450 patients with heart failure by Sin et al. demonstrated a strong association between CSA and AF. Among patients with CSA, AF was present in 23%, compared to only 12% in OSA and 7.5% in those without a breathing disorder.\textsuperscript{799} To further analyse the interaction of CSA and AF, Leung et al. examined 2,500 consecutive patients undergoing analysis of sleep, of which a sub-selection of 60 patients were diagnosed with idiopathic CSA. AF was more prevalent in the patients with idiopathic CSA (27%) compared to 60 matched individuals with OSA (1.7%) and no breathing disorder (3.3%).\textsuperscript{20} Mehra et al. further delineated this association in their “Outcomes of Sleep disorders in Older Me” (MrOS Sleep) Study.\textsuperscript{781} In total of 2,911 participants, an increasing RDI quartile was associated with increased odds of AF and complex ventricular ectopy (CVE). CSA was more strongly associated with AF (OR 2.69), whereas OSA was more strongly associated with CVE (OR 1.43), but not significantly with AF. It is likely that the association seen between CSA and AF reflects the similar pathological milieu in which they exist—namely heart failure—and is further contributed to by other factors not accounted for in the study.
(e.g. LA enlargement). However, even in the absence of heart failure (i.e. idiopathic CSA), one study has shown an increased risk of AF with CSA rather than OSA.\textsuperscript{20} The relationship may be a two-way link in that AF contributes to idiopathic CSA and/or idiopathic CSA leads to AF, or that the 2 conditions result from an unknown abnormality of central cardiorespiratory regulation.\textsuperscript{20,662,805}

Based on the current literature, it appears likely that both CSA and OSA contribute to the development of AF through some similar and some unique, pathological pathways given hypoxia is less pronounced in CSA and exaggerated negative intrathoracic pressure is absent.
1.5 RATIONALE FOR THIS THESIS AND AIMS

This thesis will present the following:

- Data demonstrating the effects of the atrial remodeling process due to the chronicity of AF.

- Data examining the effect of OSA on the presentation of patients for ablation of AF, and, how OSA affects the outcomes of this invasive therapy.

- Data characterising the atrial myocardial substrate in patients presenting for AF ablation with previously undiagnosed OSA.

- Data characterising the effects of acute sleep disordered breathing episodes on nocturnal atrial electrophysiology, which might contribute to AF.

- Data characterising the effects of hypoxia and hypercapnia on the atrial myocardium in an ex-vivo rabbit model using a microelectrode array.
2 ATRIAL SUBSTRATE REMODELING IN PAROXYSMAL AND PERSISTENT ATRIAL FIBRILLATION: SIGNAL FRAGMENTATION AND VOLTAGE

2.1 INTRODUCTION

The pulmonary veins (PV) have been identified as sources of ectopy that may trigger and also maintain atrial fibrillation (AF).\textsuperscript{5,806-808} Catheter ablation aimed at electrical isolation of these venous structures is successful in many patients with paroxysmal AF.\textsuperscript{808} However, in 30-40% with paroxysmal AF, and almost all patients with persistent AF, PV isolation as an approach is less successful and additional substrate modification is required.\textsuperscript{124,809} The ideal modality of substrate modification is still contentious.

High density mapping has demonstrated distinct electrophysiological features such as slow conduction, functional conduction block and pivot points which may be associated with fractionated potentials in the atrial substrate.\textsuperscript{228} The potential importance of such complex fractionated atrial electrograms (CFAE) in the maintenance of AF was demonstrate by Nadamenee et al., however, replication of this technique has yielded varied results.

In this prospective clinical study, we used previously validated automated software,\textsuperscript{810} to quantify the effects of arrhythmia maintenance (paroxysmal AF (PxAF) vs. persistent AF (PsAF) ) on electrogram fragmentation and its relationship to atrial voltage.
2.2 METHODS

2.2.1 STUDY POPULATION

The study comprised 36 consecutive patients presenting for AF ablation. Of these, 23 were consecutive patients with paroxysmal AF, and 13 had persistent AF. All antiarrhythmic drugs, with the exception of amiodarone, were ceased ≥5 half-lives before the study. Prior to the procedure all patients received anticoagulation with warfarin (INR 2-4) for ≥6 weeks and underwent transoesophageal echocardiography to exclude left atrial (LA) thrombus. Patients were excluded if they had a left ventricular ejection fraction <55%, significant coronary artery disease, more than physiological valvular regurgitation, valvular stenosis, or diabetes mellitus. The study protocol was approved by the institutional clinical research and ethics committee, and all patients provided written informed consent for the procedure.

2.2.2 ELECTROPHYSIOLOGICAL STUDY

The electrophysiological study was performed in the post-absorptive state under sedation with midazolam and fentanyl. A conventional transeptal puncture was performed to allow access into the LA. Anticoagulation was maintained with heparin 50 IU/kg and repeated only for procedures lasting longer than 4 hours. The following catheters were used: (i) a steerable quadripolar catheter (5mm electrode spacing, Xtrem, ELA Medical, Milan, Italy) positioned within the coronary sinus (CS) with the proximal electrode at 4 to 5 o’clock along the mitral
annulus in the left anterior oblique projection; (ii) a circumferential mapping catheter (Lasso, Biosense-Webster, Diamond Bar, USA) introduced via a long sheath (Preface, Biosense-Webster) that was continuously infused with heparinised 5% dextrose; and a 4 mm externally irrigated-tip ablation catheter (Navistar, Biosense-Webster).

Surface electrocardiograms and bipolar endocardial electrograms were continuously monitored and stored on a computer-based digital amplifier/recorder system for offline analysis (Bard Electrophysiology, Lowell MA). Intracardiac electrograms were filtered from 30 to 500 Hz and measured with computer-assisted callipers at a sweep speed of 100 mm/s.

2.2.3 ELECTROANATOMIC MAPPING

Prior to ablation, all patients underwent electroanatomic mapping using the CARTO system (Biosense-Webster) during induced or spontaneous sustained (≥10 minutes) AF. The electroanatomic mapping system has been previously described in detail; the accuracy of the sensor position has been previously validated (0.8 mm and 5 degrees). The system records the 12-lead ECG and bipolar electrograms filtered at 30-400 Hz from the mapping and reference catheters.

During AF, signals were recorded from evenly distributed points using a fill threshold of 20 mm throughout the right atrium (RA), LA and coronary sinus (CS). At each point, 5-second bipolar electrograms, together with the surface ECG were acquired. Endocardial contact during point acquisition was facilitated by fluoroscopic visualisation of
catheter motion and the distance to geometry indicated by the catheter icon on the CARTO system. Contact was confirmed on a subset with intracardiac echocardiography by visualising the catheter tissue interface (Acunav, Siemens Medical).

2.2.4 STUDY PROTOCOL

Using this system, simultaneous voltage and fragmentation maps were created of both right atrium (RA) and LA. Bipolar voltage was determined using the difference between the maximum and minimum voltage in the electrogram sequence. Electrogram fragmentation was defined as complex local electrical activity and quantified using validated software as the fragmentation index (FI). This is a quantitative description of electrogram fragmentation and is independent of the amplitude of the electrogram. By identifying the earliest deflection from baseline and the final return to baseline of the electrogram, the algorithm scores the electrogram based on the slope of each deflection. An unfragmented electrogram is given a value of 1, and an index of > 1 represents a fragmented electrogram.\textsuperscript{810}

For the purposes of evaluating regional associations, each atrium was segmented using previously validated offline software.\textsuperscript{813} The RA was segmented as the high and low lateral RA (RAHL; RALL), high and low posterior (RAHP; RALP), high and low septal RA (RAHS; RALS), and anterior RA (RAANT). The LA was segmented as posterior LA (LAPOS), LA roof (LAROF), anterior LA (LAANT), septal LA (LASEP), inferior LA (LAINF), and lateral LA (LALAT). For each region in each chamber, the mean voltage and FI was determined.
2.2.5 FOLLOW-UP

Patients were followed up with three monthly clinical history, examination, ECG and 7 day Holter monitor for 12 months, and then 6 monthly to establish freedom from AF.

2.2.6 STATISTICAL ANALYSIS

All continuous variables are reported as mean ± standard deviation or median and interquartile range as appropriate. Log transformations were performed on non-normally distributed data for statistical analysis; however, back-transformed geometric means are presented. Comparisons between means were analysed using paired or unpaired t tests as appropriate. To analyse the effects that maintenance of arrhythmia (paroxysmal versus persistent) and atrial chamber (RA versus LA) had on electrogram fractionation and voltage, given the hierarchical structure of the data, mixed linear models were constructed to account for multiple sampling within patients. Linear regression was used to examine the relationship between voltage and FI. Age, gender, hypertension and LA parasternal dimension on 2D echocardiography were treated as covariates in all models. Group comparisons at each of the atrial locations were performed and the p-values for the multiple comparisons were adjusted using Holm’s stepdown procedure. Statistical significance was established at p< 0.05. Analysis was performed using SAS v 9.1.
2.3 RESULTS

The baseline data are presented in table 1. Patients had either PxAF (n=23) or PsAF (n=13); and were undergoing electrophysiology study and ablation after having failed 5.5±1.5 anti-arrhythmic medications. There was no difference between the groups in the use of amiodarone (p=0.75). In the PxAF group, six patients had spontaneous AF at the time of presentation for ablation and the remaining 17 patients had AF induced by burst pacing which was sustained for 10 minutes before commencement of mapping. On echocardiography, expectedly, the patients with PxAF had a shorter parasternal LA dimension (43±7mm vs. 50±7 mm, p=0.01) and reduced LA volume as calculated offline using the electroanatomic maps (88±17mL vs. 124±27 mL, p<0.0001). The left ventricular ejection fraction in the PxAF group was within normal limits in both groups. An average of 102±26 points were collected per patient during electroanatomic mapping.

2.3.1 CHAMBER SIGNAL FRAGMENTATION AND VOLTAGE

The first model was designed to analyse the effects of arrhythmia maintenance, (PxAF, PsAF), atrial chamber (right, left) and their interaction, on electrogram fragmentation and voltage amplitude.

Maintenance of arrhythmia had a significant effect across both atria. Patients with PsAF had higher voltages than patients with PxAF (mean difference = 1.28mV (0.99 – 1.57), p<0.0001). LA voltages were greater than RA (mean difference = 0.87mV (0.59-1.16), p<0.0001). In this model, there was no group*chamber interaction (p=0.08). Areas of
electrical silence were recorded in 69% of PsAF patients compared to 0% of those with PxAF (p<0.0001).

Patients in the PsAF group demonstrated greater FI across both atria compared to PxAF patients (mean difference=12.042 (10.56 - 13.52), p<0.0001). LA fragmentation was greater than RA (mean difference 6.65 (5.17 – 8.13, p<0.0001). There was an interaction for group*chamber (p=0.01) suggesting differences due to arrhythmia maintenance were not constant across both atrial chambers.

2.3.2 REGIONAL SIGNAL FRAGMENTATION AND VOLTAGE

Within the RA, those with PxAF had higher voltages than those with PsAF (mean difference, 1.41mV (1.04 – 1.77 mV), p<0.0001) (figure 2.1 and 2.2). There was also a significant difference in the voltages according to regions within the RA (p<0.0001). There was no interaction between AF maintenance and region suggesting a homogeneous effect on voltages (p=0.34) Within the LA (figure 2.1 and 2.2), patients with PxAF also had higher voltages than those with PsAF (mean difference 0.83 mV (0.57 – 1.1mV), p<0.0001) and there was a significant difference in voltages within the regions (p<0.0001). Also, the effect of AF maintenance was homogeneous within the LA (p=0.9). Pairwise comparisons to assess these significant regional differences within the left atrium identified the LA posterior wall (mean voltage 1.15mV), roof (1.19mV), inferior wall (1.53mV) and septum (1.58mV) as the sites with the lowest mean voltages compared to the LA anterior (1.78mV), lateral walls (2.16mV) and LA appendage (3.6 mV) (all p<0.001 for these pairwise comparisons)
In the RA, FI was higher in PsAF compared to PxAF (mean difference, 10.13 (8.51 – 11.74), p<0.0001) (figure 2.3). There was also a significant difference amongst the regions (p=0.01). There was no significant interaction between AF maintenance and region suggesting a homogenous effect (p=0.12) of arrhythmia duration. In the LA again, PsAF patients had greater FI (mean difference 14.01 (11.55 – 16.6), p<0.0001), there were significant regional differences (p<0.0001) and the degree of change within the regions was more heterogeneous (p=0.03). Pairwise comparisons identified that regions with the highest FI were the LA posterior wall (31.70) and roof (30.11) being more fragmented than the LA inferior wall (26.8), anterior wall (25.37), septal wall (24.87), lateral wall (20.53) and appendage (17.78) (all p<0.01 for these pairwise comparisons).

2.3.3 RELATIONSHIP BETWEEN FRAGMENTATION AND VOLTAGE

To examine whether FI and voltage were related, a final model predicting voltage from FI was constructed. After correcting for covariates, significant main effects were seen for FI ($R^2 = 0.73$, p<0.0001). A scatterplot graph (figure 4) demonstrates the close relationship of log fractionation and voltage. There is clustering of data points around the lines predicted by the model.

2.3.4 FRAGMENTATION, VOLTAGE AND OUTCOME

Complete electrical isolation of the pulmonary veins (PVI) was performed on all patients, and, success was considered the first
endpoint. Multivariate logistic regression analysis to identify the relationship amongst termination of the arrhythmia study variables (FI and voltage) and covariates (gender, HT, LA parasternal diameter) identified mean LA FI to be the only predictive parameter for inability to terminate AF after PVI (OR 2.1, 95% CI 1.05-4.05, p=0.03). LA voltages were not predictive of termination of AF after PVI. Patients were followed up for 9±2 months. At the end of the study period, on multivariate logistic regression analysis, neither signal voltages nor fragmentation index were predictive of long term AF freedom, with only LA parasternal dimension remaining predictive (OR 1.12, 95% CI 1.002-1.44, p=0.049).

2.4 DISCUSSION

2.4.1 MAJOR FINDINGS

This study demonstrates the effect of arrhythmia maintenance on the electrogram signal fragmentation and voltage.

- PsAF results in greater FI and lower recorded electrogram voltages than PxAF.
- FI is greater, and voltages are lower in the LA compared to the RA.
- There is regional variation in both FI and voltage with areas of electrical silence recorded in patients with PsAF.
- There is a relationship between FI and successful termination of AF after PVI alone. Long term outcome was dependent on LA parasternal size but not FI or voltage on multivariate regression analysis.
2.4.2 COMMENT

Several studies have addressed how incorporation of complex fractionated atrial electrogram (CFAE) ablation affects immediate and long-term outcomes from AF ablation, however, not all of them have demonstrated the degree of clinical benefit obtained from the first reports of Nademanee et al.\textsuperscript{230,240,814} Underpinning this is the understanding that CFAE represent areas of altered atrial myocardium resulting in critical areas of conduction slowing, directional change or mother-rotors.\textsuperscript{122,228,815,816} Other studies have demonstrated the relationship between CFAE and ganglionated plexi located in the atria in juxtaposition to the pulmonary veins.\textsuperscript{817,818} Stiles et al., in a study on twenty patients undergoing high-density contact mapping, demonstrated a higher signal fractionation in conjunction with higher dominant frequency in patients with persistent compared to paroxysmal AF. In this study, CFAEs were noted in areas adjacent to those with high dominant frequencies.\textsuperscript{220} Fractionation, however, may not necessarily be stable within a particular area of the atrium as suggested by sheep studies demonstrating meandering rotors.\textsuperscript{231}

Indeed, in sinus rhythm studies, various substrates for atrial fibrillation, such as heart failure,\textsuperscript{6} age,\textsuperscript{205} mitral valve disease\textsuperscript{9} and paroxysmal AF\textsuperscript{10} itself, have been identified on the basis of increased signal fractionation during sinus rhythm associated with areas of low voltage in the right and left atrium. Interestingly, in a study on 12 patients with persistent AF, by Teh et al., areas of CFAE recorded during AF demonstrated normal electrophysiology during sinus rhythm suggesting
their presence might identify the presence of focal sources of activation in a region of “normal” atrial myocardium.\textsuperscript{819}

In keeping with previous studies demonstrating regional differences in electrogram voltages and fractionated signals,\textsuperscript{820,821} our study demonstrates the non-uniformity of atrial myocardial disease in atrial fibrillation with a greater emphasis on the role of the LA. We identified LA areas of reduced voltage and greater signal fragmentation – the LA roof, inferior and posterior wall. Other studies, e.g. by Park et al., using automated CFAE mapping and a NavX 3D electroanatomic mapping system identified low-voltage CFAE areas surrounded by adjacent high-voltage non-CFAE areas. These were located mostly at the septum, roof or LA appendage.\textsuperscript{822} We have shown that areas of reduced myocardial signal voltage, a surrogate marker loss of atrial myocardium or fibrosis, are also associated with increase signal fragmentation i.e. CFAE. Previous studies have identified areas of reduced voltage to be correlated with an increase in wavefront splitting and dominant frequency.\textsuperscript{823} In this study, Jacquemet et al. demonstrated that slowed conduction and a reduction in transverse coupling of cells resulted in a reduction in electrogram amplitude but not an increase in fractionation of signals, however, introduction of microfibrosis via collagenous septa resulted in marked signal fractionation that correlated with the density and length of the septa. Together these studies suggest that progression of AF from paroxysmal to persistent disease involves a highly complex substrate characterised by lower voltages and increased signal fractionation as recorded in the electrophysiology laboratory, and representing atrial fibrosis and heterogeneity of conduction on a micro-
architectural level. Furthermore, the presence of a higher FI was related to the failure of AF termination on completion of pulmonary vein isolation prior to additional substrate ablation. This suggests that in patients with greater myocardial substrate change, there is a greater reliance on additional substrate ablation to attain termination of AF.

Whilst our study demonstrated a relationship between higher baseline FI and lower voltages, only a high FI predicted failure from PVI for termination of AF. We did not determine the difference in FI of the various segments immediately before termination, nor did we target highly fragmented signals prior to completion of pulmonary vein isolation. Interestingly, Takahashi et al. demonstrated that percentage of continuous signal activity but not bipolar voltage fractionation index and local dominant frequency predicted local impact of ablation. They concluded that fractionated electrograms separated by an isoelectric segment may represent passive propagation, but, continuous activation, especially with an activation gradient from the proximal to distal electrode, may represent localised re-entry. Elayi et al. compared the impact of CFAE ablation on the prevalence of non-pulmonary vein triggers inducing AF in 2 groups of patients with long standing persistent AF. In this study, patients either underwent pulmonary vein and posterior wall isolation (PVAI) or PVAI and biatrial ablation of CFAEs i.e. biatrial ‘defragmentation’. On restoration of sinus rhythm, isoproterenol was started and the earliest non-pulmonary vein foci inducing AF in both groups was determined from ECG and intra-cardiac electrograms, correlating it with pre-ablation CFAE map created with a 3D mapping program. These non-pulmonary vein triggers were ablated until AF was
no longer inducible spontaneously or with IV isoproterenol. The major finding from this study was that, in patients with long standing persistent AF, non-pulmonary vein triggers were correlated with stable or transient CFAE in 48% and 28% respectively, and, AF ablation success rates in these patients may be improved by elimination of non-pulmonary vein triggers.\textsuperscript{525}.

Detailed mapping of the atrial myocardium prior to ablation may, therefore, not only identify areas of CFAE which may be suitable targets, but the degree of signal fragmentation may indicate the need for substrate modification addition to pulmonary vein isolation to obtain termination of AF. Interestingly, from our study, LA size was the only predictive variable for long term outcome, perhaps suggesting that modification of the substrate by maintaining a milieu that does not promote atrial dilatation, e.g. reduction in systemic blood pressure, is more important.

2.4.3 LIMITATIONS

There are several limitations to this study. Firstly, we did not take into account the length of time patients with PxAF were in AF prior to commencement of the study. We only allowed patients who entered the procedure in sinus rhythm 10 minutes in AF before mapping was commenced, hence, they may have been less of an opportunity for acute electrical remodeling. The abnormalities observed in this study are proposed to constitute the substrate predisposing to AF; however, the development of clinical arrhythmia depends on many other factors, such
as potential triggers and perpetuators. Further to this, patients were not screened for other possible substrate modifiers, such as obesity and obstructive sleep apnoea.

2.5 CONCLUSION

In this study, FI was used to characterise atrial electrograms based on their duration and complexity (independent of voltage). It allowed characterisation of the heterogeneity of atrial electrograms throughout the atria. Electrogram FI was found to be inversely related to voltage and a marker of failure of AF termination immediately after PVI. Identification of greater FI within fibrillating atria may indicate a necessity for substrate modification in addition to pulmonary vein isolation, to achieve termination of AF. However, in this study, for long term success at the completion of the study period, LA size remained the only predictive variable.
Table 2.1
Baseline characteristics of study patients

<table>
<thead>
<tr>
<th></th>
<th>PxAF</th>
<th>PsAF</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>23</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>55 ± 9</td>
<td>58 ± 7</td>
<td>0.4</td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>11</td>
<td>0.14</td>
</tr>
<tr>
<td>Duration of AF</td>
<td>17.4±30 (hours)</td>
<td>18±16 months</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>LVEF (Simpsons rule %)</td>
<td>68.2</td>
<td>57.27</td>
<td>0.01</td>
</tr>
<tr>
<td>LA parasternal dimension (mm)</td>
<td>43+/−7</td>
<td>50+/−7</td>
<td>0.01</td>
</tr>
<tr>
<td>LA volume (mL)</td>
<td>88+/−17</td>
<td>124+/−27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>9</td>
<td>6</td>
<td>0.75</td>
</tr>
</tbody>
</table>

(PxAF, paroxysmal atrial fibrillation; PsAF, persistent atrial fibrillation; LVEF, left ventricular ejection fraction; LA, left atrial)
These electroanatomic maps are taken from a study of a patient with paroxysmal AF (left) and a patient with persistent AF (right). Top figures are PA view, bottom figures are LAO view. Atria are oriented in the posteroanterior projection (top image) and left anterior oblique projection (bottom image). The colour scale is identical in both images with red representing voltages ≤0.5 mV and purple being voltage ≥5mV. Grey coloured areas represent areas of electrical silence. The patient with persistent AF has greater areas of lower voltage and areas of electrical silence compared to the higher voltages recorded in the patient with paroxysmal AF.
This figure depicts mean bipolar voltage of the right and left atrial regions derived from electroanatomic mapping.
Figure 2.3
Right and left atrial regional distribution of fragmentation index

Right atrium

This figure depicts mean fragmentation index of the right and left atrial regions derived from electroanatomic mapping.
The plot depicts the close relationship between voltage and fragmentation index with clustering of points around the predicted regression line ($R^2 = 0.73$, $p < 0.0001$).
OBSTRUCTIVE SLEEP APNOEA AND ATRIAL FIBRILLATION: ARRHYTHMIA BURDEN, PERSISTENCE AND SUCCESS OF ABLATION

3.1 INTRODUCTION

Large scale epidemiological studies have highlighted the growing prevalence of atrial fibrillation (AF). It is increasingly recognized that there is an association between obstructive sleep apnoea (OSA) and AF, and this is especially important given its association with obesity and the current ‘obesity epidemic’. Catheter ablation of AF is increasingly utilized as a treatment for symptom relief of AF, and despite the recognition that OSA is prevalent in the population of AF patients, it is not clear how many patients presenting for ablation have OSA, or the effect OSA has on the outcomes of ablation.

The current study aims to assess the prevalence of OSA in patients with AF and the characteristics of their presentation for catheter ablation. We hypothesise that patients presenting with AF may have occult OSA that may worsen the presenting symptoms, promote the persistence of the arrhythmia and ultimately affect the procedural success of catheter ablation.

3.2 METHODS

3.2.1 STUDY POPULATION

The study comprised of 90 consecutive patients with AF who were referred to our institution for consideration for first-time catheter ablation
for management of their arrhythmia. Patients were characterised according to current HRS guidelines: \(^1\) paroxysmal AF was defined as recurrent AF terminating spontaneously within seven days; persistent AF was defined as AF sustained beyond seven days or lasting less than seven days but requiring DC cardioversion; permanent AF was defined as AF with duration greater than 1 year where attempts at cardioversion have previously failed or had not been attempted.

Patients were excluded from the study for the following criteria: age <18 years or >80 years; chronic heart failure (LVEF ≤50%), valvular heart disease (assessed by echocardiography as being more than physiological), previous myocardial infarction, untreated hypertension, or diabetes. All patients provided written informed consent for the study protocol, which was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital, Adelaide, Australia.

To gauge the mean respiratory apnoea/hypopnoea index (AHI) in the community, we recruited 20 consecutive volunteers from the same catchment area to undergo overnight polysomnography (PSG) and Epworth Sleepiness Scale assessment. Inclusion criteria for these patients included freedom from cardiovascular disease, good exercise tolerance and absence of any symptoms suggesting AF.

### 3.2.2 SLEEP STUDY

All patients underwent evaluation for sleep disordered breathing (SDB) home polysomnography (PSG) using a portable Somté device (Compumedics, Victoria, Australia). The Somté device allows continuous monitoring of the electroencephalogram, thoracic and
abdominal effort via respiratory conductive plethysmography, facilitated by chest and abdominal bands, nasal airflow via nasal cannulae recording the nasal pressure signal, arterial oxygen saturation by finger pulse oximetry, limb movement and body position and continuous ECG (lead II). The use of portable devices has been validated against in-hospital PSG for the diagnosis of SDB.\textsuperscript{603}

All sleep studies were analysed and scored by a single experienced trained sleep scientist (Board of Registered Polysomnographic Technologists) and reported by a single sleep physician. Scoring reliability was regularly checked against other scorers in the laboratory and scorers in other laboratories in Australia and New Zealand through an inter-laboratory scoring concordance program. Sleep was scored according to the criteria of Rechtschaffen and Kales,\textsuperscript{600} micro-arousals from sleep according to American Sleep Disorders Association criteria\textsuperscript{829} and respiratory events using “Chicago” criteria.\textsuperscript{830} Apnoeas were scored if there was a complete cessation of airflow for 10 seconds or more, and, hypopnoeas were scored if the event was 10 seconds or more in duration and accompanied by either (a) a clear decrease $>50\%$ in a valid measure of breathing during sleep or (b) a clear reduction in breathing amplitude during sleep that was associated with either an oxygen desaturation of $>3\%$ or an arousal. An apnoea was categorised as obstructive if there was sustained respiratory effort in the chest/abdominal bands, central if respiratory effort was reduced by at least $90\%$ for at least $90\%$ of the duration of the event. The diagnosis of OSA was made on the basis of the AHI; AHI$<15$
was classified as mild, $15 \leq \text{AHI} < 30$ was considered moderate and $\text{AHI} \geq 30$ established the presence of severe OSA.

To evaluate symptoms related to OSA, daytime sleepiness was determined by the Epworth Sleepiness Scale.\textsuperscript{529}

### 3.2.3 ATRIAL FIBRILLATION SYMPTOMATIC BURDEN

The University of Toronto symptom severity scale (AFSS, table 1),\textsuperscript{831-833} a disease-specific measure of quality of life in AF allowing for subjective and objective measures of arrhythmia burden, was employed to quantify the burden of arrhythmia. The scale included questions under the categories of event “frequency”, “longest duration” and “symptom severity”. Each of the three categories was scored on a scale from 0 to 10 and hence a final severity score was allocated with highest scores representing the most severe presentations.

### 3.2.4 ELECTROPHYSIOLOGY STUDY AND ABLATION

The electrophysiology study, previously described\textsuperscript{10}, was performed in the post-absorptive state with intravenous midazolam for sedation and fentanyl for analgesia. To summarise, conventional transeptal puncture allowed access to the left atrium (LA) for a circular mapping catheter (Biosense Webster, Diamond Bar, U.S.A) and 3.5mm tip externally-irrigated ablation catheter (Biosense Webster). Intravenous heparin (100IU/kg initial dose) was used to maintain an ACT of 300-350 seconds. Radiofrequency ablation was performed with power of 30W, temperature 48°C and normal-saline irrigation rates of 30 mL/minute.
Ablation was guided by non-fluoroscopic electroanatomic navigation. All patients underwent wide circumferential ablation of the pulmonary veins with an end-point of electrical isolation. Additional substrate modification in the form of linear ablation (roofline and/or mitral isthmus) or CFAE ablation was performed in patients with an episode of AF ≥48 hours or with a LA size ≥57mm (longest diameter). Cavotricuspid isthmus ablation with an endpoint of bidirectional isthmus block was performed only in patients with a history of typical flutter.

3.2.5 FOLLOW-UP

Study patients were followed up at 3 monthly intervals for 12 months and 6 monthly thereafter the index procedure, until recurrence (which ever occurred first). At all reviews, each patient underwent evaluation with clinical history, physical examination, ECG and 7 day ambulatory Holter monitoring. Antiarrhythmic medications were continued in the pre and post-ablation period with medications terminated at 6 weeks by the treating physician. Warfarin was prescribed pre-ablation according to the CHADS2 score.1 Post- ablation, all patients continued on warfarin for a minimum of 12 months. Procedural success was determined as the absence of atrial arrhythmias lasting >30 seconds, after observation of a 3 month post-ablation blanking period.

3.2.6 STATISTICAL ANALYSIS

Continuous variables are reported as mean ± standard deviation (SD) or median and interquartile range (IQR) as appropriate. Categorical variables used were gender, hypertension, diabetes mellitus, and AF
persistence. Continuous variables included age (years) and body mass index (BMI) calculated as kilograms divided by height in meters squared (kg/m\(^2\)). LA area (cm\(^2\)) was calculated on pre-ablation 2D echocardiography and corrected for body surface area (cm\(^2\)/m\(^2\)).

Study sample characteristics were grouped according to severity of OSA and were compared using the Chi square test for categorical variables and ANOVA for continuous variables. Univariate and multivariate linear regression was used to evaluate the relationship between OSA and AF burden. To study the relationship between OSA and the persistence of AF, univariate and multivariate binary logistic regression analysis was used. Covariates included age, BMI, LA size indexed to BSA, hypertension, diabetes mellitus and AF persistence.

To determine the relationship between OSA and AF recurrence post-procedure, patients were divided into either none/mild (AHI<15) or moderate/severe OSA (AHI ≥15) and Kaplan-Meier curve constructed. Secondly, time-to-event Cox proportional hazard regression method was used to study the individual relationship of OSA and the aforementioned risk factors.

Statistical tests were performed using SPSS16 (SPSS Inc, Chicago, Illinois) and a p<0.05 was considered significant.
3.3 RESULTS

3.3.1 STUDY POPULATION BASELINE CHARACTERISTICS

Baseline characteristics are presented in table 3.2. On categorical analysis of the 90 patients, 59 (65.5%) had paroxysmal AF, 26 (29%) had persistent AF and 5 (5.5%) had permanent non-cardiovertable AF.

Severe OSA was diagnosed in 19 (21%), moderate OSA in 27(30%), mild or no OSA was diagnosed in 44 (49%). The mean AHI for the cohort was 19±16 and mean central AHI (CAHI) 1.1±3.6. By definition, the number of sleep related breathing events, including the number of CSA events were more in the severe OSA group. In this analysis, the presence and severity of the CSA was not considered clinically relevant, and when included did not alter the outcomes.

Patients were on a variety of antiarrhythmic medications although their usage for similar amongst the groups. There was no difference in the presence of hypertension, diabetes mellitus and non-obstructive coronary artery disease amongst the groups. There was a trend towards an increase in the history of stroke with severe OSA (p=0.05). Indexed left atrial areas were larger in patients with at least moderate OSA. Patients had normal systolic function on inclusion criteria, and there was no difference in the left ventricular diastolic measurement E/E’.

3.3.2 COMMUNITY SAMPLE COMPARISON TO STUDY POPULATION

Compared to the community sample of individuals without AF or cardiovascular disease, patients with symptomatic AF presenting for
ablation were older (51±10 vs. 58±11, p=0.007), had similar gender mix (p=0.6), had a greater BMI (24.5±2.6 kg/m² vs. 30.8±8.6 kg/m², p=0.002) and neck circumference (36.8±3.4cm vs. 40.5±4 cm, p<0.001). The mean AHI was higher (7±5 vs. 19±16 p<0.001), however, the mean ESS was similar (5±3 vs. 6±3, p=0.18). Univariate logistic regression demonstrated that, in the presence of at least moderate OSA (AHI ≥15), the odds ratio (OR) for AF was 9.4 (2.0-42.9, p=0.004). On multivariate analysis when including age, BMI, diabetes and hypertension, moderate OSA remained predictive of AF with OR 6.3 (1.2 – 32.6, p=0.03).

3.3.3 OSA AND AF SEVERITY

On a univariate analysis of variance, for AFSS as a measure of AF burden, there was significant difference between the groups (p<0.001) (Figure 1). Linear regression (table 3.3) demonstrated the significant relationship between AF severity and univariate predictors, of which at least moderate severity OSA (AHI ≥15) remained significant on multivariate analysis.

3.3.4 OSA AND AF PERSISTENCE

When AF was considered as a categorical variable for persistence, in a model including risk factors for AF, the only significant predictor was a diagnosis of at least moderate OSA (OR 3.38 (95% CI, 1.32-10.2), p=0.03) (table 3.4). Other measurements of OSA (i.e. average and lowest oxygen saturations (awake and asleep) were not significant predictors in either model (data not shown).
3.3.5 OSA AND AF RECURRENCE

Of the 90 patients, 76(84%) underwent AF ablation. Fourteen patients did not agree to an ablative approach, or were continued on medical therapy beyond the timeframe of the study period. One patient died from a respiratory illness before undergoing ablation. Patients undergoing ablation were followed up for a median of 12 (6.2-24) months. Thirty six (47%) remained free of recurrence while off anti-arrhythmic medication after a single ablation procedure. Of the remaining 40 patients, 29 underwent a second procedure and 4 had a procedure pending at completion of the study period. After 26±11 months and 1.4±0.5 procedures, 72% of the patients undergoing ablation were free from AF.

Kaplan-Meier analysis demonstrated that patients with a higher grade of OSA suffered recurrence at earlier time points after the index ablation procedure (p=<0.001 by log-rank test) (figure 3.2).

Severe OSA (HR 2.38 (95% CI, 1.2 - 5.06), p=0.02) and AF persistence (2.5 (95% CI, 1.22-5.3), p=0.01) were the only factors predictive of AF recurrence by Cox regression modelling after multivariate adjustment for all the aforementioned risk factors (table 3.5).

3.4 DISCUSSION

3.4.1 MAJOR FINDINGS

This study utilised overnight polysomnography to diagnose OSA in patients with symptomatic AF presenting for consideration of AF ablation. As a group, these patients did not demonstrate significant
symptoms relating to OSA as defined by the Epworth Sleepiness Scale; however, OSA had significant impact on their presentation and outcomes.

The key findings in this study were, that in symptomatic patients presenting with AF for ablation:

- OSA is highly prevalent, with at least moderate OSA in greater than half of patients.
- OSA is associated with increased burden of arrhythmia as measured by the AFSS.
- OSA is associated with persistence of AF.
- OSA is influential in the recurrence of AF after the index ablation procedure, independent of LA size and AF persistence.

3.4.2 COMMENT

The first reports of the relationship between OSA and cardiac arrhythmias date back over 25 years. More recently, Stevenson et al. demonstrated the high prevalence of sleep disordered breathing in a relatively young population of patients with paroxysmal and persistent AF. Compared to a matched population of patients without AF, 62% vs. 28% had an AHI > 15 suggesting a greater proportion with at least moderate sleep disordered breathing. On logistic regression analysis, after adjusting for relevant covariates, the odds ratio for AF was 3.04 (95% CI, 1.24 -7.46, p=0.02). In a report by Jongnarangsins et al 324 AF patients with paroxysmal or permanent disease were retrospectively examined. A diagnosis of OSA was established in 10% of the study
population prior to their ablation procedure. The numbers were small because not every patient underwent polysomnography for the diagnosis of OSA prior to their procedure. Despite this, on multivariate analysis, OSA was the strongest predictor of recurrent AF after catheter ablation (OR 3.04, 95% CI 1.11-8.32, p=0.03). This was independent of BMI, which failed to demonstrate significance in the final analysis. More recently, Chilukuri et al. has published two reports relating BMI, OSA to catheter ablation outcomes for AF. In the first study, 210 patients with AF were investigated with the Berlin Questionnaire to obtain a high risk score suggesting a diagnosis of OSA. Of these patients, 44% returned a high-risk result and, on multivariate analysis, this was identified as an independent predictor of failure after catheter ablation for AF (OR 4.53 CI: 1.21-16.67, p=0.02).

A meta-analysis recently published during the time this thesis was being prepared, included the two above studies and 4 others and concluded that OSA diagnosed on polysomnography was considered a strong predictor of risk of AF recurrence (RR 1.40, p=0.0004). Our study performed polysomnography in all patients undergoing ablation, and multivariable adjusted analysis again demonstrated the significant relationship with OSA severity with relationship to AF burden, AF persistence and AF recurrence after ablation.

The literature now has several reports of potential modifies to the atrial substrate resulting from the pathophysiological sequelae of OSA. These potential mechanisms include swings in intra-thoracic pressure causing an increase in LA volume, changes in autonomic tone, intermittent hypoxia, oxidative stress, and inflammation all of
which may contribute to AF triggers and maintenance. Whilst our study, like others, demonstrates a poorer arrhythmia freedom from AF ablation, we have not examined the impact of CPAP on these outcomes. The suggestion is that CPAP will improve ablation outcomes in a similar way it improves maintenance of sinus rhythm after cardioversion, by reducing OSA negative remodeling and removing potential triggers.

Why patients with OSA may be more symptomatic, however, is not clear from this study. Given the non-exclusivity of AF symptoms, there are likely to be some symptoms shared by the two conditions e.g. lethargy. Also, paroxysms or AF or loss of AF rate control may be more symptomatic in patients with other associated manifestations of OSA such as diastolic dysfunction. A small but interesting study by Yu et al. in patients with AF, normal left ventricular systolic function and an atrial defibrillator, showed that even short episodes of AF resulted in a reduction in left ventricular systolic and diastolic parameters. Furthermore, diastolic function appeared to normalise quicker, within hours, but systolic function took longer to recover, up to 1 week. AF episodes lasting >48 hours suppressed systolic function more than shorter duration episodes. In our patients, we used E/E’ to characterise left ventricular diastolic dysfunction, however, there was no difference detected on this measure between the groups.

Given these findings, management of OSA with continuous positive airways pressure (CPAP) may result in an improvement of symptoms associated with AF, a reduction in potential pathological atrial modifiers and perhaps reduce the requirement for AF ablation in this population of patients. For those patients with OSA undergoing ablation
for AF, treatment with CPAP may improve freedom from arrhythmia outcomes.

3.4.3 LIMITATIONS

Symptoms of AF are subjective and hence there are various demographic and psychological reasons why patients might understate or overstate their symptoms. Whilst the AFSS provides a framework of objective and subjective measures to allow characterisation of symptom severity and duration, it is limited in its accuracy by the inherent subjectivity of patients. Furthermore, it is sometimes difficult to determine whether patients’ symptoms at a time were in fact due to AF. We limited echocardiographic evaluation of LV diastolic function to a simple but reasonably robust measurement of diastolic performance (E/E’) but this may not have been sufficient to differentiate abnormalities in diastology between the groups examined.

3.5 CONCLUSION

Patients with AF have a significant amount of undiagnosed OSA that contributes to their presentation, both in terms of severity and persistence. Moreover, the presence of severe OSA has a definite impact on failure rates after first AF ablation. It remains to be shown whether treating this modifiable risk factor with CPAP has an impact on the burden of AF, the presentation for AF ablation and the arrhythmia free outcomes after ablation.
Atrial fibrillation severity scale (AFSS) allows for subjective and objective measures of AF burden with each category scored from 1 to 10. (AF, atrial fibrillation).

<table>
<thead>
<tr>
<th>Severity Parameter</th>
<th>Descriptor</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AF frequency</strong></td>
<td>Less than once per year</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>About once per year</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>About 2-4 times per year</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>About once per month</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>About twice per week</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>About once per week</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>About 2-3 times per week</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>About 4-5 times per week</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>About once per week</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>More than once per day</td>
<td>10</td>
</tr>
<tr>
<td><strong>AF duration</strong></td>
<td>A few minutes</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Less than 30 minutes</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>30-45 minutes</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>About 1 hour</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Between 1-4 hours</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Between 5 and 12 hours</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Between 13 and 24 hour</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>About 1-3 days</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>About 4-7 days</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>&gt;7 days</td>
<td>10</td>
</tr>
<tr>
<td><strong>AF severity</strong></td>
<td>Most recent episode</td>
<td>1-10</td>
</tr>
<tr>
<td></td>
<td>1= not at all</td>
<td></td>
</tr>
<tr>
<td></td>
<td>to</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10= extremely severe</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1
Modified AFSS algorithm for scoring AF symptoms\textsuperscript{831-833}
Table 3.2
Study population characteristics and polysomnography data

<table>
<thead>
<tr>
<th>Study population characteristics</th>
<th>AHI &lt; 15 n=44</th>
<th>15 ≤ AHI &lt; 30 n=27</th>
<th>30 ≤ AHI n=19</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHI</td>
<td>8±4.1</td>
<td>19.7±3.9</td>
<td>44.8±12.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CAHI*</td>
<td>0.4±0.4</td>
<td>0.8±1.7</td>
<td>1.5±2</td>
<td>0.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.8±4.3</td>
<td>31.3±5.3</td>
<td>31.6±6.2</td>
<td>0.06</td>
</tr>
<tr>
<td>Neck circumference (cm)</td>
<td>39.5±4.1</td>
<td>41±3.3</td>
<td>42.4±2.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Paroxysmal AF</td>
<td>34(77)</td>
<td>16(59)</td>
<td>11(58)</td>
<td>0.2</td>
</tr>
<tr>
<td>Persistent AF</td>
<td>10(33)</td>
<td>11(41)</td>
<td>8(42)</td>
<td>0.2</td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta blockers n(%)</td>
<td>10(23)</td>
<td>13(48)</td>
<td>7(37)</td>
<td>0.8</td>
</tr>
<tr>
<td>Class I antiarrhythmic</td>
<td>11(25)</td>
<td>3(11)</td>
<td>3(16)</td>
<td>0.2</td>
</tr>
<tr>
<td>Class III antiarrhythmic</td>
<td>21(47)</td>
<td>10(27)</td>
<td>12(63)</td>
<td>0.2</td>
</tr>
<tr>
<td>Statins</td>
<td>11(25)</td>
<td>9(33)</td>
<td>6(32)</td>
<td>0.7</td>
</tr>
<tr>
<td>ACEI/ARBs</td>
<td>20(45)</td>
<td>16(59)</td>
<td>12(63)</td>
<td>0.33</td>
</tr>
<tr>
<td>LA size* (cm²/m³)</td>
<td>11(9.5-13)</td>
<td>13(11-17)</td>
<td>13(11-14)</td>
<td>0.04</td>
</tr>
<tr>
<td>LV posterior wall thickness (cm)</td>
<td>1±0.1</td>
<td>1.1±0.1</td>
<td>1.1±0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>E/E'</td>
<td>10±3.4</td>
<td>9.6±3.3</td>
<td>10.1±2</td>
<td>0.9</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3(7)</td>
<td>2(7)</td>
<td>2(10)</td>
<td>0.8</td>
</tr>
<tr>
<td>Hypertension</td>
<td>16(36)</td>
<td>16(59)</td>
<td>11(58)</td>
<td>0.1</td>
</tr>
<tr>
<td>Non-obstructive CAD</td>
<td>2(5)</td>
<td>2(7)</td>
<td>3(16)</td>
<td>0.3</td>
</tr>
<tr>
<td>Stroke</td>
<td>2(5)</td>
<td>1(4)</td>
<td>4(21)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

| Polysomnography data            |              |                   |              |    |
| Total sleep time                | 379±81       | 382±61            | 381±72       | 0.9   |
| Sleep efficiency                | 78±14        | 75±11             | 77±13        | 0.7   |
| % NREM sleep                    | 78±8         | 76±6              | 78±8         | 0.6   |
| Median average SaO₂(%)          |              |                   |              |    |
| NREM                            | 93.8±1.6     | 93.4±1.6          | 92.3±1.9     | 0.01  |
| REM                             | 93.6±2.1     | 93±2              | 91.1±4.1     | 0.01  |
| Median lowest SaO₂(%)           |              |                   |              |    |
| NREM                            | 89.1±3.8     | 87.8±2.5          | 84.1±4.8     | <0.001 |
| REM                             | 86.3±15      | 86.2±4.2          | 83±8         | 0.6   |
| Epworth sleepiness score        | 6.1±4        | 5.7±3.1           | 6.7±2.2      | 0.6   |

(AHI, apnoea-hypopnoea index; CAHI, central apnoea-hypopnoea index; BMI, body mass index; ACEI/ARB’s, angiotensin converting enzyme inhibitor/angiotensin receptor blocker; LA, left atrial; LV, left ventricular; NREM, non rapid eye movement; REM, rapid eye movement; SaO₂, oxygen saturation; BSA, body surface area)
Table 3.3
Multivariable-adjusted regressions of risk factors, OSA and AF burden

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Univariate (95% CI)</th>
<th>p</th>
<th>Multivariable-adjusted (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.06 (0.005-0.124)</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>0.26 (-1.1 - 1.6)</td>
<td>0.71</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.04 (-0.092-0.163)</td>
<td>0.58</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>LA size*</td>
<td>0.15 (0.03 - 0.34)</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.7 (0.6-2)</td>
<td>0.29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.29 (-1.14 - 3.7)</td>
<td>0.29</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>AF persistence</td>
<td>1.81 (0.5 - 3.13)</td>
<td>0.01</td>
<td>1.54 (0.2 - 2.89)</td>
<td>0.03</td>
</tr>
<tr>
<td>AHI ≥ 15</td>
<td>2.69 (1.5-3.9)</td>
<td>&lt;0.001</td>
<td>2.14 (1.3-3.7)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

* indexed to BSA

(BMI, body mass index; AHI, apnoea-hypopnoea index; BSA, body surface area)
### Table 3.4
Multivariable-adjusted odds ratios of risk factors, OSA and AF persistence

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Univariate (95% CI)</th>
<th>p</th>
<th>Multivariable-adjusted (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.001 (0.9-1.04)</td>
<td>0.96</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gender</td>
<td>1.003 (0.4-2.5)</td>
<td>0.99</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BMI</td>
<td>1.023 (0.8-1.1)</td>
<td>0.59</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LA size*</td>
<td>1.1 (1 - 1.27)</td>
<td>0.18</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.54 (0.6-3.7)</td>
<td>0.33</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.29 (0.03-2.6)</td>
<td>0.27</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AHI ≥ 15</td>
<td>3.57 (1.4-9.1)</td>
<td>0.01</td>
<td>3.38 (1.32-10.2)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*B indexed to BSA

(BMI, body mass index; AHI, apnoea-hypopnoea index; BSA, body surface area)
Table 3.5
Multivariable-adjusted hazard ratios of risk factors, OSA and AF recurrence

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Univariate (95% CI)</th>
<th>p</th>
<th>Multivariable-adjusted (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.02 (1.0-1.06)</td>
<td>0.96</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>0.95 (0.50-1.90)</td>
<td>0.99</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>1.0 (1.0-1.09)</td>
<td>0.59</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>LA size*</td>
<td>1.08 (1 - 1.15)</td>
<td>0.04</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.18 (0.61-3.27)</td>
<td>0.33</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.29 (0.60-4.51)</td>
<td>0.27</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>AF persistence</td>
<td>1.98 (1.06 - 3.7)</td>
<td>0.03</td>
<td>2.5 (1.22 - 5.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>AHI ≥ 15</td>
<td>2.37 (1.26-4.46)</td>
<td>0.01</td>
<td>2.38 (1.2-5.06)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

* indexed to BSA

(BMI, body mass index; AHI, apnoea-hypopnoea index; BSA, body surface area)
This box and whiskers graph demonstrates the distribution of patients’ University of Toronto Severity Scale scores (AFSS) according to severity of AHI (AHI<15, mild; 15 ≤AHI<30, moderate; AHI ≥30 severe). There was a significant difference in the scores amongst the 3 groups.
Kaplan-Meier curves showing the proportion of patients who maintained sinus rhythm after a single ablation procedure according to the severity of OSA. (AHI, apnoea-hypopnoea index)
4 ELECTROANATOMIC REMODELING IN OBSTRUCTIVE SLEEP APNOEA

4.1 INTRODUCTION

Obstructive sleep apnoea (OSA) is increasingly recognized as a potential risk factor for the development of atrial fibrillation (AF).\textsuperscript{19,786,789} In addition, OSA has been associated with a greater risk of recurrence of AF after cardioversion\textsuperscript{787} and catheter ablation.\textsuperscript{791} Despite the enormity of the problems associated with the epidemic of AF, the mechanisms by which OSA predisposes to the development or recurrence of AF remains unknown. Recent evidence has highlighted the importance of atrial structural and electrical remodeling in the substrate predisposing to AF.\textsuperscript{6,7,9,10,196,197,205,219} We postulated that patients with OSA have evidence of atrial structural and electrical remodeling to account for their predisposition to AF.

4.2 METHODS

4.2.1 STUDY POPULATION

The study comprised 40 patients undergoing first time ablation of paroxysmal AF. These patients were dichotomized by polysomnography (PSG) into OSA (moderate to severe OSA: Apnoea-Hypopnoea Index [AHI] \( \geq 15 \); \( n=20 \)) and a reference group (none to mild OSA: AHI<15; \( n=20 \)). Patients with significant central sleep apnoea (CSA) were excluded from the study. A total of 96 consecutive patients were screened to obtain 40 patients fulfilling the study criteria. To avoid the
remodeling due to arrhythmia itself, patients were excluded if they had AF during the week prior or arrhythmia >30secs on continuous monitoring within 48 hours of ablation. We also excluded patients with chronic heart failure (LVEF ≤50%), valvular heart disease (assessed by echocardiography as being more than physiological), previous myocardial infarction, untreated hypertension, diabetes, and those on amiodarone or digoxin. Patients without OSA were matched on the basis of age and BMI category (normal, overweight, obese). AF burden was quantified using the previously validated University of Toronto Atrial Fibrillation Severity Scale (AFSS).\textsuperscript{831} All anti-arrhythmic medication was ceased ≥5 half-lives prior to the study. All patients provided written consent to the study protocol approved by the Human Research Ethics Committee of the Royal Adelaide Hospital, Adelaide, Australia.

4.2.2 SLEEP STUDY

Portable PSG (Somté, Compumedics, Victoria, Australia) was used to assess for the presence of OSA.\textsuperscript{603} All sleep studies were scored by a single certified sleep scientist and reported by a single sleep physician. Sleep was scored according to the criteria of Rechtschaffen and Kales,\textsuperscript{600} micro-arousals from sleep according to American Sleep Disorders Association criteria\textsuperscript{829} and respiratory events using “Chicago” criteria\textsuperscript{830}. Apnoea were defined by a reduction of at least 90% in airflow measured from nasal pressure transducer which lasted at least 10 seconds and accompanied by either (a) a clear decrease >50% in a valid measure of breathing during sleep or (b) a clear reduction in breathing amplitude during sleep that was associated with either an oxygen
desaturation of >3% or an arousal. An apnoea was categorised as obstructive if there was sustained respiratory effort in the chest/abdominal bands, and central if respiratory effort was reduced by at least 90% for at least 90% of the duration of the event.

4.2.3 ELECTROPHYSIOLOGICAL STUDY AND ABLATION

The electrophysiological study was performed in the post-absorptive state with minimal conscious sedation utilizing midazolam and fentanyl, allowing the patient to maintain an adequate airway and oxygen saturation >95%. The left atrium was accessed via transeptal puncture and biatrial electroanatomic mapping carried out immediately before radiofrequency ablation. Electrophysiological evaluation was performed after ablation to avoid induction of sustained AF.

Following the study protocol, patients with AF underwent circumferential pulmonary vein ablation with an end-point of electrical isolation. Additional substrate modification in the form of linear ablation (roofline and/or mitral isthmus) or CFAE ablation was performed in patients with an episode of AF ≥48 hours or with a left atrial (LA) size ≥57mm (longest diameter). Cavotricuspid isthmus ablation with an endpoint of bidirectional isthmus block was performed only in patients with a history of typical flutter.

4.2.4 STUDY PROTOCOL

Atrial electrophysiological study was performed by positioning multi-polar catheters (Daig Electrophysiology): (i) a 10-pole catheter (2-5-2 mm inter-electrode spacing) within the coronary sinus (CS) with the
proximal bipole at the CS ostium as determined in the best septal left anterior oblique view; (ii) a 20-pole “Crista” catheter (1-3-1 mm inter-electrode spacing) positioned along the crista terminalis with the distal tip superiorly located such that the second bipole lay at the junction of the SVC and the RA. The catheter was stabilized in position using a long sheath (CSTA) to ensure contact with the crista terminalis; (iii) a 20-pole catheter (2-5-2 mm inter-electrode spacing) was placed along the lateral RA (LRA); and (iv) a roving 4-pole mapping catheter was used to perform measurements at the high-septal RA (HSRA).

Electrophysiological evaluation included the following:

Atrial effective refractory period (ERP), the longest coupling interval failing to propagate to the atrium, was determined at the following sites: high (HLRA) and low LRA (LLRA); proximal (PCS) and distal CS (DCS); and the HSRA. The mean was evaluated from three measurements at twice diastolic threshold at cycle lengths of 600 and 450ms using an 8-beat drive followed by an extra-stimulus, starting with a coupling interval of 150ms increasing in 10ms increments. If AF was induced lasting > 5 mins, no other data was collected. ERP heterogeneity was determined by the coefficient of variation of ERP (SD/mean x 100%).

Atrial conduction was determined along linearly placed catheters by pacing the distal bipole (1,2) and determining the conduction time to bipole 9,10 at the LRA and the CS. Mean conduction time was determined over 10 consecutive beats during constant pacing at 600 and 450 ms. Lead II P wave duration (PWD) was determined as an indicator of bi-atrial conduction.
Site-specific conduction abnormalities were determined at the crista terminalis. The number of catheter bipoles with discrete double potentials separated by an isoelectric interval or fractionated signals of $\geq 50$ms duration (collectively called complex electrograms), and the maximum electrogram duration were determined during constant pacing and for the shortest-coupled captured extra-stimulus from either side of this structure (PCS and LLRA).

The mean of three corrected sinus node recovery time (CSNRT) measurements was determined using a 30-second drive train at cycle lengths of 600 and 450ms, correcting for the baseline cycle length.

Electroanatomic maps were created of both atria during sinus rhythm using the CARTO mapping system and a 3.5mm tip catheter (Navistar, Biosense-Webster). The electroanatomic mapping system has been previously described $^{6,7,9,10,219}$ Endocardial contact during point acquisition was facilitated by electrogram stability, fluoroscopy and the catheter icon on the CARTO system. Points were acquired in the auto-freeze mode if the stability criteria in space ($\leq 6$mm) and local activation time ($\leq 5$ms) were met. Mapping was performed with an equal distribution of points using a fill-threshold of 15mm. Offline, local activation time was manually annotated to the peak of the largest amplitude deflection on bipolar electrograms; in the presence of double potentials this was annotated at the largest potential. If the bipolar electrogram displayed equivalent maximum positive and negative deflections, the maximum negative deflection on the simultaneously acquired unipolar electrogram was used. Points not conforming to the surface ECG P wave morphology
or <75% of the maximum voltage of the preceding electrogram were excluded.

For evaluating regional voltage differences, each atrium was segmented using previously validated offline software\textsuperscript{9,10}. The RA was segmented as the HLRA, LLRA, high- (HPRA) and low-posterior RA (LPRA), HSRA and low-septal RA (LSRA), and anterior RA (ARA). The LA was segmented as posterior LA (POST), anterior LA (ANT), septal LA (SEPT), inferior LA (INF), lateral LA (LAT) and LA roof (ROOF). Low voltage points were defined as $\leq 0.5\text{mV}$ and electrically silent points as $\leq 0.05\text{mV}$ (the noise level of the system).

Isochronal activation maps (5ms intervals) of the atria were created and regional conduction velocity determined in the direction of the wave-front propagation (least isochronal crowding). An approximation of conduction velocity was determined by expressing the distance between two points as a function of the difference in local activation time. Mean conduction velocity for each region was determined by averaging the conduction velocity between 5 pairs of points, as previously described\textsuperscript{9,10}.

Complex electrograms were defined as (i) fractionated Signals—multiple intersections of the baseline of $\geq 50\text{ms}$ duration; and (ii) double Potentials—two discrete potentials separated by an isoelectric interval with a total duration of $\geq 50\text{ms}$.

**4.2.5 STATISTICAL ANALYSIS**

Continuous variables are reported as mean ± SD (or median and interquartile range as appropriate). Categorical variables are reported as
number and percentage. Proportions were compared using the Fisher exact test. Unpaired $t$ tests were used to compare means between the two groups when normally distributed. Comparisons with adjustment for multiple sampling within patients and nesting of data were performed using a mixed linear model for continuous data and a generalised estimating equation for categorical variables. Covariates corrected for in the mixed model included BMI, LA volume indexed to body surface area (BSA), and University of Toronto Atrial Fibrillation Severity Score (AFSS). Statistical tests were performed using SPSS version 16. Statistical significance was set at a value of $P<0.05$.

4.3 RESULTS

4.3.1 BASELINE CHARACTERISTICS

Baseline characteristics are summarised in Table 1. There were no significant differences in the use of beta blockers ($p=0.3$), calcium channel blockers ($p=0.5$), or ACE inhibitors/ARB’s ($p=0.7$) between the groups. Patients with OSA demonstrated significantly larger atria indexed to BSA ($p=0.009$). Left ventricular function was normal for all patients. University of Toronto Severity Scale (AFSS) duration and frequency scores were similar between the two groups.

4.3.2 POLYSOMNOGRAPHY

Table 1 summarizes the findings related to the sleep studies. BMI and neck circumference were similar. Both groups had low Epworth Sleepiness Scale scores suggesting they were minimally symptomatic.
from OSA. Patients in the OSA group had a mean AHI >30 suggesting a moderate to severe degree of the breathing disorder.

4.3.3 ELECTROPHYSIOLOGY STUDY

4.3.3.1 ATRIAL REFRACTORINESS

At each site and at each CL there was no difference in the ERP between patients with significant OSA and the reference group; at CL 600ms: 226±82 versus 228±54 ms (p=0.9) and at CL 450ms 205±72 versus 200±63ms (p=0.7). ERP was longer at a CL of 600ms compared to a CL of 450ms for both groups of patients (p<0.001 for both CL’s), indicating the preservation of physiological rate-adaption of ERP. Similarly, there was no difference in the heterogeneity of ERP at 600ms, 11±5 versus 12±3% respectively (p=0.7) and at 450ms, 15±9% versus 12±4% respectively (p=0.2)

4.3.3.2 ATRIAL CONDUCTION TIME

The mean atrial conduction time was significantly longer in patients with OSA (CS/LRA: 51±7/58±10 ms versus 45±10/51±10; p=0.03). Mixed effect analysis revealed no significant interaction between patient group and the region of measurement (p=0.8) suggesting homogenous conduction slowing in OSA patients compared to those without OSA. This conduction slowing was further accentuated by the shortest conducted S2; 101±17 versus 89±19 ms (p=0.03); again with homogenous slowing of atrial conduction (p=0.8).

P wave duration was also significantly longer in patients with OSA compared to those without OSA; 138±8 versus 120±15 ms (p=0.01).
4.3.3.3 SITE-SPECIFIC CONDUCTION ABNORMALITIES AT THE CRISTA TERMINALIS

Site-specific conduction abnormalities at the crista terminalis were more numerous in patients with OSA (5±1 versus 2±2; p=0.003) and of greater duration (62±11 versus 44±19 ms; p=0.03). As illustrated in Figure 4.1, OSA had great number and duration of complex electrograms during the drive train (p=0.001 and p<0.001, respectively) and the premature stimuli (p=0.003 and p=0.003, respectively).

4.3.3.4 SINUS NODE FUNCTION

OSA patients demonstrated evidence of sinus node remodeling with statistically longer CSNRT than reference patients (CL=600: 397±113 versus 266±73 ms (p=0.02) and CL 450ms: 380±100 versus 240±69 ms; (p=0.006)).

4.3.3.5 ELECTROANATOMIC MAPPING

An average of 184±44 points per patient were examined in the LA and RA with no difference in the number of points between both groups.

4.3.3.6 VOLTAGE ABNORMALITIES

Patients with OSA demonstrated significantly larger left (116±35 versus 83±11 mL; p=0.01) and right atria (111±28 versus 87±18 mL; p=0.02) (Figure 4.2).

The bipolar voltage was substantially reduced in both the RA (1.6±0.2 versus 2.4±0.1mV; p<0.001) and LA (1.5±0.1 versus 2.4±0.2mV; p<0.001) in patients with OSA (Figure 4.3). Mixed linear
modelling demonstrated a significant difference in the various regions with regard to bipolar voltage for patients with OSA (RA p=0.001; LA p=0.02). In the RA, but not the LA, there was significant interaction between the region of measurement and patient grouping suggesting a heterogeneous difference in voltage change (p=0.03). Patients with OSA were more likely to have a mean regional voltage <1 mV (OR 6.2, 95% CI 2.3 to 16.8) compared to those without OSA after accounting for covariates of age, gender, BMI, LA volume indexed to BSA and AFSS. In addition, while none of the patients in the reference group demonstrated regions of electrical silence, 7 patients with OSA had areas of atrial voltages <0.05mV (p=0.008) predominantly within the LA.

4.3.3.7 CONDUCTION ABNORMALITIES

Patients with OSA demonstrated significantly slower mean conduction velocity in both the RA (0.8±0.1 versus 1.2±0.1 mm/ms; p<0.001) and LA (0.9±0.1 versus 1.2±0.1 mm/ms; p<0.0001). Mixed linear modelling demonstrated a significant effect of OSA (p<0.001) with homogenous slowing of conduction with no specific regional effect. Regional differences in conduction velocity are presented in Figure 4.4.

4.3.3.8 COMPLEX FRACTIONATED ELECTROGRAMS

Patients with OSA had a significantly greater number of points with complex electrograms compared to those in the reference group in both the RA (22±10 versus 13±5%; p=0.02) and LA (25±8 versus 16±7%; p=0.01). Whilst these points were distributed throughout both
atria, clustering was noted particularly along the LA roof and septum, and along the crista terminalis within the RA.

4.4 DISCUSSION

4.4.1 MAJOR FINDINGS

This study utilized detailed electrophysiologic and electroanatomic mapping to characterise electrophysiological and electroanatomical atrial abnormalities in patients with OSA. Those with OSA demonstrated the following compared to reference patients:

- Structural change with increased atrial size and extensive areas of low voltage and regions of electrical silence, perhaps suggesting loss of atrial myocardium, fibrosis or underlying conduction dissociation.
- Conduction abnormalities characterised by longer P-wave duration, prolonged conduction times, site-specific conduction abnormalities and greater regions of atrial complex electrograms.
- Prolongation of CSNRT suggesting sinus node remodeling.
- No difference in ERP in the resting state.

These findings suggest that OSA portends a significantly more diseased atrial substrate, thus contributing to the development and maintenance of AF.
4.4.2 COMMENT

Guilleminault et al. in 1983 reported on the relationship between OSA and cardiac arrhythmias in 400 patients, reporting that 48% of patients with OSA had cardiac arrhythmias during the night. Indeed, the prevalence of nocturnal paroxysms of AF was more than 3% compared to 0.4%-1% in the general population. In patients with AF, Gami et al. extended these observations using the Berlin questionnaire for diagnosis of OSA and found a high prevalence (49%) amongst patients undergoing electrical cardioversion of AF compared to general cardiology patients without AF where the incidence of OSA was 32%. Multivariate regression revealed an odds ratio of 2.2 highlighting the association between OSA risk and AF. The same group also published a large retrospective cohort of 3542 Olmsted County adults without previous AF who had undergone an initial diagnostic polysomnography within the 20 year study period. AF occurred in 133 of these subjects resulting in the cumulative probability of developing AF of 14% during follow-up of up to 15 years. On univariate analysis, OSA was a significant independent predictor with a hazard ratio of 2.18. Various measures of OSA severity were also found to be relevant and most interestingly, lowest nocturnal oxygen saturation, demonstrated a significant HR of 3.08 (per 1 unit decrease in logarithm). The decrease in nocturnal oxygen saturation remained significant in the multivariate model in subjects <65 years of age. More recently, Stevenson et al. prospectively examined 90 patients with paroxysmal and persistent AF and 45 age-matched controls and reported that 62% of the AF patients had OSA compared to 38% of controls (p=0.01). Mehra et al. demonstrated a 4 fold higher odds of
nocturnal complex arrhythmias in patients with sleep disordered breathing after adjusting for potential confounders and there is a temporal relationship of triggering of these events to respiratory disturbances as recently described. To add to the complexity of the effects of sleep disordered breathing, studies have shown a markedly increased prevalence of atrial fibrillation among patients with CSA in the absence and presence of congestive cardiac failure, as well marked effects on AV nodal electrophysiology likely resulting from autonomic modulation of the AV node in heart failure patients. In addition to these studies that have demonstrated a greater frequency of OSA in patients with AF, others have demonstrated that OSA may be a predictor of failure of a rhythm control strategy. Kanagala et al. studied the recurrence rates for AF after cardioversion and observed a much higher rate of recurrence in patients OSA. Similarly, emerging data suggest that the recurrence rates after AF ablation may also be higher.

Atrial remodeling and the substrate predisposing to AF have been studied in a number of chronic conditions that are associated with the development of AF. Li and Nattel demonstrated that the remodeling that leads to the substrate for AF was different to that due to arrhythmia itself. In this experimental model of chronic heart failure, these investigators observed no change or increase in ERP but a greater inducibility of AF due to heterogeneous conduction associated with atrial fibrosis. Similar structural changes have also been implicated in experimental models of mitral regurgitation, hypertension, and other causes of non-ischemic cardiomyopathy. Human studies in chronic heart failure, sinus node disease, mitral stenosis, atrial septal
defects, and even lone AF have all demonstrated marked electroanatomic changes with evidence of loss of atrial myocardium with/without area of electrical silence which were associated with conduction abnormalities with no change or an increase in ERP.

The current study demonstrates remarkably similar changes in this population of patients with OSA and AF studied remote from the episode of arrhythmia. Compared to reference patients with no or mild OSA, and in the absence of significant other structural heart disease, these individuals had evidence of a more extensive atrial substrate which is likely to be the substrate accounting in part for the development and progression of AF in OSA.

There are several potential pathophysiological contributors which may predispose a patient with OSA to develop AF. OSA is increasingly recognized to be associated with cardiovascular conditions such as heart failure and hypertension. These conditions are established risk factors for the development of AF. In our study, there were no patients with untreated hypertension, and no difference between the groups for patients with treated stable hypertension. In addition, we excluded patients with any significant structural heart disease. Thus these factors are not likely to have contributed to the changes that we observed.

There is evidence to implicate a multifactorial direct effect of OSA on the atria. Potential mechanisms include swings in intra-thoracic pressure causing an increase in left atrial volume, autonomic changes, intermittent hypoxia and oxidative stress, and inflammation, all of which have been variably implicated as causes of
fibrosis., and thus, important mechanisms involved in chronic structural remodeling of the human atrium.

Two important recent animal studies have progressed our knowledge on the acute electrical remodeling secondary to hypercapnia\(^{200}\) and that of vagal activation of AF due to negative tracheal pressure\(^{202}\) which is suppressible by combined \(I_{Kr}\) and \(I_{Kur}/I_{to}\) blockade.\(^{201}\) It is plausible that repetitive changes to atrial electrophysiology may have long term detrimental effect on the myocardium, although the exact mechanisms are currently unknown.

Finally, in the current study, patients with OSA had significantly larger atria than those without OSA. This finding raises the potential role of stretch, an established stimulus for the substrate for AF, in the mechanisms of AF in OSA.

This study provides mechanistic insight into the link between OSA and AF. It confirms significant atrial remodeling as a result of OSA. However, importantly, these patients did not have traditional symptoms of OSA to any greater extent than controls. As a modifiable risk factor, these findings suggest that OSA should be routinely screened for in patients with AF.

Verma et al.\(^{845}\) have previously demonstrated that areas of atrial electrical silence are powerful independent predictors of long-term recurrence after pulmonary vein antrum isolation for AF. Thus the presence of OSA, with its associated remodeling, may predict procedural failure, as highlighted by a recent meta-analysis.\(^{791}\) To minimise recurrence and the need for a repeat procedure, extensive atrial mapping for scar-related pro-arrhythmic isthmuses and areas that could
contribute to the maintenance of AF may be necessary to improve the success of ablation in patients with OSA. Furthermore, evidence suggests a reduction in arrhythmia burden with therapy directed at OSA,\textsuperscript{19,787} as well as a potential positive remodeling effect suggested by a reversal of atrial dilatation,\textsuperscript{691} and amelioration of the deleterious effects of inflammation and oxidative stress on the endothelium.\textsuperscript{846} Hence, given the mechanistic link suggested by this study, OSA should be routinely screened for and treated ‘upstream’ in conjunction with other AF management strategies in attempts to provide positive atrial remodeling and improvement of arrhythmia free outcomes.

### 4.4.3 LIMITATIONS

AF is a complex disease with complex interactions between triggers and, perpetuators and substrate. While we have made attempts at avoiding the remodeling due to arrhythmia by excluding patients with recent arrhythmic episode, we cannot exclude a potential additive effect of rate-related remodeling from previous episodes. However, prior studies have demonstrated no additive effects of episodic AF and resolution of electrical remodeling within days.\textsuperscript{847}

There are limitations in our ability to determine atrial conduction velocity in ambulatory human subjects. Therefore we have used both electroanatomic mapping and linear catheter placement that provided similar corroborative findings. Indeed, these surrogates for atrial conduction are previously published and well established as methodology in examining clinical substrates for AF.\textsuperscript{6,9,10,205}
4.5 CONCLUSION

Patients with OSA demonstrate significant atrial remodeling characterised by structural abnormalities with a reduction in atrial myocardial voltage regions of electrical silence, conduction slowing and sinus node dysfunction. These factors may account for the development and maintenance of AF in patients with OSA.
Table 4.1
Baseline characteristics

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<th>Reference (n=20)</th>
<th>OSA (n=20)</th>
<th>p</th>
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<tbody>
<tr>
<td>Male</td>
<td>16</td>
<td>17</td>
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</tr>
<tr>
<td>Age</td>
<td>51±12</td>
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</tr>
<tr>
<td>BMI</td>
<td>29±3.5</td>
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<tr>
<td>Neck circumference</td>
<td>41±4</td>
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<tr>
<td>Hypertension (treated)</td>
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<td>11</td>
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<tr>
<td>Structural heart disease</td>
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<td>0</td>
<td>1</td>
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<tr>
<td>AFSS – frequency</td>
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**Echocardiography Data**

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<td>9.2±1.1</td>
<td>9.6±1.1</td>
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<td>Estimated RV systolic pressure</td>
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<td>IVSd (cm)</td>
<td>1.09±0.1</td>
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<tr>
<td>LVPWd (cm)</td>
<td>1.1±0.07</td>
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<td>LVEDD (cm)</td>
<td>5.2±0.2</td>
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<td>3.1±0.4</td>
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<tr>
<td>LVEF (%)</td>
<td>62±1</td>
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**Polysomnography Data**

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<tr>
<td>Sleep efficiency (%)</td>
<td>78±3</td>
<td>80±2</td>
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<tr>
<td>Mean AHI</td>
<td>6±3</td>
<td>38±22</td>
<td>&lt;0.0001</td>
</tr>
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<td>Mean CAI</td>
<td>1±1.2</td>
<td>2.6±2.5</td>
<td>0.09</td>
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<td>25±14</td>
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<td>Epworth sleepiness scale</td>
<td>7 (4 - 9)</td>
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(BMI – Body mass index; BSA – Body surface area; AFSS – Atrial fibrillation severity scale; LA – left atrial; IVSd -interventricular septum diameter); LVPWd - left ventricular posterior wall diameter; LVEDD -left ventricular end-diastolic dimension; LVESD - left ventricular end-systolic dimension; LVEF - left ventricular ejection fraction; AHI - apnoea hypopnoea index; CAI – central apnoea index)
Figure 4.1
Site-specific conduction abnormalities at the crista terminalis

During the drive train (S1) and the shortest coupled interval (S2), there were a greater number of complex atrial electrograms present at the crista terminalis in OSA patients. (OSA, obstructive sleep apnoea; PCS, proximal coronary sinus; LLRA, low lateral right atrium)
Figure 4.2
Electroanatomic voltage maps of the right and left atria

Figure legend overleaf
Figure legend 4.2

Representative electroanatomic voltage maps from the right and left atria (RA; LA) oriented in left anterior oblique projection. They demonstrate the differences between a representative patient with OSA (AHI=86; top) and a reference (AHI<15; bottom). Colour scale is set to “low voltage” (≤0.5 mV) and high voltages (purple) ≥5mV. There are greater areas demonstrating low voltage signals (and electrical silence (grey)) as well as complex signals (double and fractionated signals) in the OSA patient. There are greater signals demonstrating conduction abnormalities evidenced by complex signals (double potentials and fractionated potentials) in the OSA patient.
Mean (± standard deviation) bipolar voltage of the right atrial and left atrial regions from the electroanatomical maps. (OSA, obstructive sleep apnoea; HLRA, high lateral right atrium; LLRA, low lateral right atrium; HSRA, high septal right atrium; LSRA, low septal right atrium; HPRA, high posterior right atrium; LPRA, low posterior right atrium; ARA, anterior right atrium; POST, posterior left atrium; ANT, anterior left atrium; SEPT, septal left atrium; INF, inferior left atrium; LAT, lateral left atrium, ROOF, roof of left atrium)
Mean (± standard deviation) conduction velocities of the right atrial and left atrial regions from the electroanatomic maps. (OSA, obstructive sleep apnoea; HLRA, high lateral right atrium; LLRA, low lateral right atrium; HSRA, high septal right atrium; LSRA, low septal right atrium; HPRA, high posterior right atrium; LPRA, low posterior right atrium; ARA, anterior right atrium; POST, posterior left atrium; ANT, anterior left atrium; SEPT, septal left atrium; INF, inferior left atrium; LAT, lateral left atrium, ROOF, roof of left atrium)
NOCTURNAL ALTERATIONS OF ATRIAL ELECTROPHYSIOLOGY IN OBSTRUCTIVE SLEEP APNOEA: EVIDENCE FOR ACUTE ELECTRICAL REMODELING

5.1 INTRODUCTION

Sleep disordered breathing (SDB) is common and affects up to 20% of the population. It is associated with significant cardiovascular morbidity including hypertension, myocardial ischemia, stroke and arrhythmias and sudden cardiac death. More recently, including, obstructive sleep apnoea (OSA) and central sleep apnoea (CSA) have been increasingly recognised as potential risk factors for the development of atrial arrhythmias including atrial fibrillation (AF). In addition, OSA has been associated with a greater recurrence of AF after cardioversion and catheter ablation. Several mechanisms of these arrhythmias have been proposed including the evolution of, or, contribution to hypertension, heart failure and coronary artery disease, the effect of hypoxaemia via its effects on the autonomic nervous system, systemic inflammation, oxidative stress, negative intrathoracic pressures, cardiac structural changes such as left atrial stretch and left ventricular diastolic dysfunction, and vascular endothelial dysfunction.

Recently, Monahan et al. evaluated the temporal relationship between respiratory events in OSA and the onset of paroxysmal and non-sustained ventricular tachycardia and concluded that the relative risk
of paroxysmal AF and non-sustained ventricular tachycardia increased in the vulnerable period after a respiratory disturbance, irrespective of the association with hypoxia or cortical arousals.\textsuperscript{788}

This study, therefore, sought to characterise the acute electrical remodeling that occurs at night in patients with sleep disordered breathing relative to the respiratory disturbance and hypoxia observed in obstructive apnoeas and hypopnoeas.

\section*{5.2 METHODS}

\subsection*{5.2.1 SLEEP STUDY}

The study protocol was approved by institutional research and ethics committees, and all participants gave written and informed consent. Consecutive patients undergoing electrophysiological evaluation and ablation for AF were screened prior to their procedure for sleep disordered breathing with home polysomnography (PSG) (Somté, Compumedics, Victoria, Australia). The Somté device allows continuous monitoring of the electroencephalogram, thoracic and abdominal effort via respiratory conductive plethysmography facilitated by chest and abdominal bands, nasal airflow via nasal cannulae recording the nasal pressure signal, arterial oxygen saturation by finger pulse oximetry, limb movement and body position. The use of portable devices has been validated against the gold standard in-hospital PSG for the diagnosis of sleep disordered breathing.\textsuperscript{603} All sleep studies were analysed and scored by a single experienced trained sleep scientist (Board of Registered Polysomnographic Technologists) and reported by a single
sleep physician. Standard definitions of respiratory events were used for analyses as per recommendations of the American Academy of Sleep Medicine. Sleep was scored according to the criteria of Rechtschaffen and Kales, microarousals from sleep according to American Sleep Disorders Association criteria and respiratory events using “Chicago” criteria. Apnoeas were scored if there was a complete cessation of airflow for 10 seconds or more and hypopnoeas were scored if the event was 10 seconds or more in duration and accompanied by either (a) a clear decrease >30% in a valid measure of breathing during sleep or (b) a clear reduction in breathing amplitude during sleep that was associated with either an oxygen desaturation of >3% or an arousal. Central apnoeas were scored if there was no breathing effort detected. Patients were eligible for enrolment in the study if the apnoea-hypopnoea index (AHI) was >15 events/hour.

Patients were excluded if they had symptomatic AF during the week prior, or arrhythmia >30 secs by continuous monitoring in the 48 hours prior, to their procedure. Patients with chronic heart failure (defined as EF <45% ± New York Heart Association >I), more than physiological valvular regurgitation on 2D echocardiography, previous myocardial infarction or coronary revascularisation procedure, untreated hypertension, endocrine disease including diabetes or prior amiodarone usage. All anti-arrhythmic medication was ceased ≥5 half-lives prior to the study.
5.2.2 ABLATION PROCEDURE

Each electrophysiological study was performed in the post-absorptive state with conscious sedation utilizing minimal midazolam and fentanyl to keep the patient comfortable.

At the beginning of the procedure, a 20 polar (5-2-5-60-5-2-5mm spacing, St Jude Medical, St Paul, Minnesota, USA) was advanced to the coronary sinus (CS) via a right jugular vein 7F sheath. It was positioned so that the distal group of bipoles sat securely in the coronary sinus with the proximal pair of electrodes from that group positioned at the coronary sinus ostium. The proximal ten bipoles were thus positioned to record lateral right atrial electrograms (figure 51).

The LA was accessed via transeptal puncture. Following this, anticoagulation was maintained with repeated bolus injections of unfractionated heparin to maintain an activated clotting time of 300-350 seconds. Bolus doses of midazolam and fentanyl were used to obtain conscious sedation during the procedure. The effects of these medications were reversed with flumazenil and naloxone bolus injections at completion of the procedure.

Patients underwent circumferential pulmonary vein ablation with an end-point of isolation confirmed by mapping (Lasso, Biosense-Webster, Diamond Bar, USA). Ablation was performed using a delivered power of 30W with irrigation rates of 30/min (Navistar, Biosense-Webster). Additional substrate modification including cavitricuspid isthmus ablation, coronary sinus ablation or complex fractionated electrogram ablation was not performed.
At completion of the procedure, the 7F sheath was secured to the skin and the extra-luminal catheter firmly taped to the patient’s thorax to prevent inadvertent movement during the time spent recumbent at night. On transfer to the coronary care unit, the distal and proximal electrode pairs of the 20 polar catheter were connected to a portable electrophysiology recording computer (LabSystem Pro, Bard Electrophysiology, Lowell, MA, USA). This was positioned behind a privacy screen in the patient’s room. Somté PSG recording equipment was then connected to the patient in a similar manner as described above. The two computer systems were time synchronised for simultaneous recording and subsequent offline analysis.

5.2.3 POST-PROCEDURE STUDY PROTOCOL

The study protocol required patients to undergo post-procedural nocturnal electrophysiological evaluation in the coronary care unit after their ablative procedure, whilst undergoing simultaneous overnight portable polysomnography. During sleep, atrial electrophysiology was assessed from the proximal coronary sinus (CS). Programmed electrical stimulation was performed with an 8 beat pacing drive trains (S1; cycle lengths (CL) 600ms and 450ms) at twice diastolic threshold. Atrial extrastimuli were introduced decrementally to establish effective refractory period (ERP) defined as the longest S1-S2 interval failing to evoke an atrial depolarisation. This was repeated throughout periods of non-rapid eye movement (NREM) stage II sleep. Atrial conduction time (CT) was calculated in two directions; 1) from the proximal CS to distal CS electrogram, and, 2) from the proximal CS electrogram to distal right
atrial lateral wall (LAT) electrogram. This was performed during the drive train (CT_{CS-S1} and CT_{LAT-S1}) and during the shortest coupled extrastimulus (CT_{CS-S2} and CT_{LAT-S2}). Local conduction delay (CD), i.e. the time interval between A1A2 after subtracting the corresponding S1S2 (CD = A1A2-S1S2) was measured at the distal coronary sinus (CD_{CS}) and lateral RA (CD_{LAT}) bipoles in response to premature extrastimuli from the proximal coronary sinus, as previously described.\textsuperscript{214,859}

To evaluate the effect of respiratory disturbance on atrial electrophysiology, ‘study periods’, previously described by Monahan et al. (called ‘hazard periods’) based on data from the Sleep Heart Health Study and other physiological/clinical studies, were identified.\textsuperscript{470,729,788,860-862} These periods were defined as episodes of respiratory disturbance with an associated reduction in SpO\textsubscript{2} $\leq$ 92% lasting up to 90 seconds. In our study, these study periods were classified as either obstructive (OA) or hypopnoea (HY) events according to the definitions given above. The components of the study period were 1) a period of respiratory disturbance of $\sim$20-30 seconds before oxygen desaturation (period 1), 2) a period of $\sim$15-30 seconds including maximum oxygen desaturation or nadir (period 2), 3) a period of recovery from hypoxia lasting $\sim$20-30 seconds (period 3). Recordings were also performed during wakefulness with stable breathing for three consecutive 30 second periods (i.e. not associated with episodes of oxygen desaturation, apnoea or hypopnoea). These were used as reference recordings for comparisons.
5.2.4 STATISTICAL ANALYSIS

Continuous variables are reported as mean ± standard deviation or median and interquartile range as appropriate. Degree of change (Δ) in a given variable was examined between periods 1 and 2 (period_{1-2}), periods 2 and 3 (period_{2-3}) and between periods 3 and 1 (i.e. the residual change at the end of an event compared to the beginning; period_{3-1}). An example of this is: \( \Delta \text{SaO}_2 \) between periods 1 and 2 = \( \frac{(\text{SaO}_2(\text{period } 1) - \text{SaO}_2(\text{period } 2))}{\text{SaO}_2(\text{period } 1)} \). Logarithmic transformation was performed on all non-normal data for entry into the statistical model. Comparisons with adjustment for multiple sampling within patients were performed using a mixed linear model. For categorical analysis of directionality of the changes, a ±10ms change in ERP and ±5ms change in conduction time and delay were considered significant for each period transition. Comparison in categorical data were made using Chi square. Linear regression was used to assess the relationship between desaturation and study variables. Statistical tests were performed using SPSS version 16 (SPSS Inc, Chicago, Illinois) and statistical significance was set at a value of \( p<0.05 \).

5.3 RESULTS

Fifty-nine patients who attended the laboratory for electrophysiological evaluation and ablation for paroxysmal AF underwent portable polysomnography. Twenty-six patients had moderate to severe OSA. Of these, 7 patients consented for the overnight study. The baseline demographic data, echocardiographic measurements and polysomnographic study results are presented in table 1. One patient
developed AF at the beginning of the study protocol (during ERP testing) and was excluded as no further information could be derived and neither DC cardioversion, nor delivery of antiarrhythmics was allowed as part of study protocol. This patient was subsequently cardioverted to sinus rhythm the following day.

The mean age of the patients was 59±5 years and the mean BMI was in the obese range. The patients all had a normal left ventricular ejection fraction and were NYHA class I. Their left atria were mildly dilated. The mean AHI suggested the patients had severe OSA. CSA events were present in a small number; however, the nocturnal events were predominantly obstructive in nature.

5.3.1 POLYSOMNOGRAPHIC DATA

From the polysomnographic data, 60 study events (30 OA and 30 HY) and 30 REF events were selected. These corresponded with appropriately timed electrophysiological data of good quality signals without movement artefact. The mean oxygen saturation for period_1 (SaO_2), i.e. prior to oxygen desaturation, was 96.5±6% for REF events, 95.7±2% for OA events, and 95.2±1.2% for HY events. For REF events, the ΔSaO_2 for period_1-2 was 0.3±0.4%. By comparison, the ΔSaO_2 for period_1-2 were 7.8±3.7% (p<0.001) for OA events, and 4.6±2 (p<0.001) for HY events. For REF events, the ΔSaO_2 for period_2-3 was 0.5±0.5%. By comparison, ΔSaO_2 for period_2-3 for the study periods were 7.4±3.8% (p<0.001) for OA and 4.0±2% (p<0.001) for HY events.
5.3.2 EFFECTIVE REFRACTORY PERIOD

For HY and OA events, absolute ΔERP was significant for period \(_1\), \(_2\), \(_2-3\), as well as period \(_3-1\) when compared to the changes observed in REF events (table 5.2). Figure 5.2 represents each study event relative to its period 1 ERP. This was irrespective of pacing cycle length (p values for the interaction were non-significant in all cases). The OA events demonstrated a greater absolute ΔERP-period \(_1-2\) than HYP events (p=0.007). The ΔERP-period \(_2-3\) was similar for HY and OA events (p=0.2). The ΔERP-period \(_3-1\) was also similar between OA and HY events. (p=0.13).

For period \(_1-2\) and period \(_2-3\), the percentages of events that resulted in a ≥10ms ΔERP are depicted in Figure 5.5. For OA and HY events, there was tendency to a negative change for period \(_1-2\), however some events exhibited a positive change, and some no change. The same occurred in period \(_2-3\), with a tendency towards either an increase in ERP, but with some events decreasing or no change. When analysing all REF and study events, the OR for a ±10ms change in ERP with period 2 was 6.2 (95% CI, 2.5-15.2; p<0.001). The proportion of events displaying a significant positive change in ΔERP-period \(_1-2\) was higher in the OA compared to HY events (37% vs. 17%, p=0.002). A similar proportion (50%) demonstrated a negative change in ERP. Univariate linear regression to assess if the degree of change was determined by the degree of hypoxia revealed a statistically significant but weak relationship (R\(^2\) = 0.06, p=0.02). This suggests other factors besides the degree of hypoxia play significant roles in determining the ERP changes.
5.3.3 CONDUCTION TIME AND CONDUCTION DELAY

For HY and OA events, there were significant changes in absolute $\Delta CT$ and $\Delta CD$ for period1-2, period2-3 and period3-1 when compared to REF events. Figure 5.3 and 5.4 represents each study event relative to its period 1 measurement. For HY events, except for $\Delta CT$ for period1-2, all other comparisons to REF events were significant for S1 and S2 conducted beats. For OA events, $\Delta CT$ and $\Delta CD$ (S1 and S2) were significant for all comparisons to REF events. This was irrespective of pacing cycle length (p values for the interaction were non-significant in all cases).

Comparing HY and OA, for CT$_{CS}$, $\Delta$CT$_{CS}$S1-period1-2 differed in the absolute magnitude the OA events demonstrating a greater degree of change than HY events ($p<0.001$). Comparing for $\Delta$CT$_{CS}$S1-period2-3, OA events also had a greater degree of change ($p=0.015$). The $\Delta$CT$_{CS}$S1-period3-1 was more marked for OSA ($p=0.001$), suggesting a greater degree of residual change at the end of an OA event. For conduction along the lateral RA, the $\Delta$CT$_{LAT}$S1-period1-2 was greater for OA compared to HY events ($p=0.01$). The $\Delta$CT$_{LAT}$S1-period2-3 and $\Delta$CT$_{LAT}$S1-period3-1 were similar between the SDB types ($p=0.2$ and $p=0.3$, respectively). The degree of change in $\Delta$CT$_{CS}$S1 and $\Delta$CT$_{LAT}$S1 were not homogenous. For OA events, $\Delta$CT$_{CS}$S1 was greater than $\Delta$CT$_{LAT}$S1 for period1-2 ($p=0.004$), but not for HY events where there was a similar degree of change. There was greater residual change at the end of a study period with $\Delta$CT$_{CS}$S1-period3-1 being larger than $\Delta$CT$_{LAT}$S1 ($p=0.006$) in OA events, however, this was less pronounced in HY events.
\( \Delta CT_{CS-S2} \) and \( \Delta CT_{LAT-S2} \) were all significant compared to REF events for period\(_{1-2} \), period\(_{2-3} \) and period\(_{3-1} \). However, there was no significant difference between the changes observed in OA and HY events. For S2 beats, \( \Delta CT_{CS-S2} \) was greater than \( \Delta CT_{LAT-S2} \) for period\(_{1-2} \) (p=0.008). There was also significant heterogeneity in period\(_{2-3} \) between regions with \( \Delta CT_{CS-S2} \) being greater than \( \Delta CT_{LAT-S2} \) in the OA events (p=0.008), and HY events (p=0.04). This was again the case for period\(_{3-1} \) with greater residual change in \( \Delta CT_{CS-S2} \) compared to \( \Delta CT_{LAT-S2} \) for both OA (p=0.02) and HY (p=0.005) events.

For \( \Delta CD_{CS} \) and \( \Delta CD_{LAT} \), significant differences were seen for all periods when compared to REF events. For period\(_{1-2} \) OA events demonstrated greater \( \Delta CD_{CS} \) (p=0.003) and \( \Delta CD_{LAT} \) (p<0.001) then HY events. There was a similar degree of change between OA and HY events for \( \Delta CD_{CS} \)-period\(_{2-3} \) (p=0.16) and \( \Delta CD_{LAT} \)-period\(_{2-3} \) (p=0.09). In OA events, for period\(_{3-1} \), there was a greater degree of \( \Delta CD_{CS} \) (p=0.007) and \( \Delta CD_{LAT} \) (p=0.01).

Categorical analyses of the SDB events (based on the percentage of least a ±5ms change in CT, and the directionality of the change are displayed Figure 5.5. When analysing all REF and study events, the OR for a significant change in \( CT_{CS-S1} \) or \( CT_{LAT-S1} \) for period\(_{1-2} \) was 12.9 (95% CI, 5.7-28.4; p<0.001). The OR for a significant (≥5ms) change in \( CT_{CS-S2} \) or \( CT_{LAT-S2} \) for period\(_{1-2} \) was 11.5 (95% CI, 5.7– 23.1; p<0.001). The proportion of events demonstrating a significant slowing in conduction during S1 and S2 were greater for OA compared to HY events (CS, p<0.001; LAT, p<0.001). For the study events, there was there was no significant relationship between measurements of conduction and the
degree of hypoxia in period 2 on univariate linear regression. This suggests other mechanisms related to the pathological consequences of hypoxia are more important than the degree of hypoxia itself.

5.4 DISCUSSION

5.4.1 MAJOR FINDINGS

This study has demonstrated significant changes to atrial electrophysiology resulting from SDB events. In summary,

- SDB events result in significant changes to ERP. The magnitude of these changes is greater with OA than HY events. Fifty percent of episodes demonstrated a negative change in ERP during the period of hypoxia; however, of remaining events, OA events were more likely to demonstrate a significant positive change in ERP.

- A greater number of events showed a slowing of conduction, and this was more marked with OA events. Some events however did show faster conduction during period 2, again demonstrative of the heterogeneity of conduction abnormalities.

- There is minimal relationship of these changes to the degree of hypoxia on regression analysis. This suggests that other dynamic factors may be more important in contributing these changes. However, hypoxia is a common factor to both hypopneas and apnoeas and is still likely to be important as outlined below.
5.4.2 COMMENT

In normal individuals without SDB, NREM sleep comprises about 85% of adult sleep. During sleep, autonomic changes ensue, with a progressive decline in sympathetic output and an increase in parasympathetic tone. The result is state of haemodynamic calm and cardiac electrical stability as demonstrated by the reduction in ventricular premature beats in sleeping subjects. In patients with SDB, this quiescence is interrupted by recurrent adverse respiratory events.

The ERP changes from the beginning of an event, through the nadir of oxygen desaturation and into recovery. This was recorded on the majority of occasions as decrease in the ERP; however, an increase in ERP was notable in OA events. Some events did not exhibit a significant change in ERP during hypoxia. The considerable variability and weak relationship to hypoxia suggested that several other factors not recorded during the experiment may have been responsible for these changes, such as activation of SNS/PNS. Conduction time, both during the drive train (S1) beat and the shortest coupled conducted beat (S2) appeared to lengthen initially during period 2 then in the latter stages shorten with recovery during period 3. However, again, these changes were variable with some conduction time shortening and sometimes, no immediate change was measured. Most prolongation of conduction time was seen with OA events. This was associated with an increase in the local CD. Notwithstanding the possibilities discussed in the limitations second, the heterogeneity in conduction likely represents the very complex pathophysiology underpinning SDB.
Together, these findings suggest that SDB acutely affects the atrial substrate in humans. The resultant changes in ERP and conduction times may predispose patients to the development of atrial arrhythmias throughout the night by acting as a trigger for ectopy, or, creating a suitable substrate that promotes reentry.

In 1983, Guilleminault et al. presented data on the relationship between OSA and AF in an observational report on 400 adults with moderately-severe OSA undergoing nocturnal assessment.\(^\text{19}\) AF was present in 3% of the population as compared to 0.4%-1.0 % of the general population. Leung et al., identified patients with idiopathic CSA from a sample of 2500 patients undergoing polysomnography.\(^\text{20}\) With single lead nocturnal analysis for arrhythmias, they compared the 60 patients that met the inclusion criteria for idiopathic CSA to 60 patients with OSA and 60 patients without SDB. Interestingly the prevalence of AF in patients with predominantly CSA was 27%, 16 fold higher that in the OSA group (1.7%) and 8 times the control group without SDB (3.3%). This lead to the conclusion that neither the apnoea-related hypoxias, nor the exaggerated negative intrathoracic pressures contributed to the higher prevalence of AF in the CSA group. Their suggestion was hypocapnia resulting from hyperventilation secondary to respiratory control instability might impair cardiac stability.\(^\text{803}\) Also, increased chemoresponsiveness,\(^\text{863}\) similar to that seen in heart failure,\(^\text{864,865}\) may indicate an underlying abnormality of the central SNS, and this may predispose to AF. Mehra et al. in the “Sleep Heart Health Study” (SHHS) demonstrated a pronounced prevalence of AF (4.8% vs 0.9%) and non-sustained ventricular tachycardia and complex ventricular
ectopy in patients with SDB after adjusting for confounders. On a subset from the SHHS, Mehra et al. also studied the ECG data of 2911 sleep studies (‘MrOS Sleep’ Study). CSA appeared more strongly associated with AF than did OSA (even after multiple adjustments and for self-reported heart failure symptoms, CSA still portended up to a 5 times increased odds for AF). Interestingly, OSA, but not CSA, was associated with nocturnal complex ventricular ectopy. There also appeared to be a threshold effect in that the increased risk of nocturnal AF was observed at a threshold AHI of 24 events/hour.

The relationship between CSA and AF has been postulated to be either AF predisposing to idiopathic CSA, or, idiopathic CSA predisposing to AF, or that both CSA and AF are secondary to another abnormality of central cardiorespiratory regulation. Respiratory control stability is an important mechanism involved in this complex relationship with an endpoint of hypocapnia affecting cellular electrical stability. Other possibilities accounting for the potential triggering of AF by CSA is central and peripheral chemoresponsiveness, which may be related to abnormalities in the central SNS. Whilst hypoxia results from both OSA and CSA, the apnoea related reduction in oxygenation is often worse in patients with OSA. In our study, as mentioned, there was, at most, only mild CSA in this group of patients. Specifically, CSA events were not analysed, however, whether this affected the observations made in OSA events is not clear.

An important study by Monahan et al. recently demonstrated that the relative risk of a paroxysmal episode of AF or non-sustained ventricular tachycardia is increased shortly after a respiratory
disturbance (OR 14.2), regardless of whether events were associated with hypoxia below 92% or an arousal. Also, the majority of arrhythmias occurred during NREM sleep and the durations of recorded arrhythmias were short – 7 seconds for paroxysmal AF, with only one long episode of 5 minutes. The results from Monahan et al., when analysed in the absence of respiratory events, but in the presence of hypoxia or arousals were unremarkable (and not reported in their paper). Apart from the single patient that had an immediate induction of AF following ERP testing, there were no other episodes of arrhythmia amongst the 6 patients studied, likely due to the fact that these patients had just undergone pulmonary vein isolation. In this study, we only examined respiratory events marked by a hypoxia and a respiratory disturbance.

OSA is defined by the interruption of airflow in the face of ongoing respiratory effort, whereas, in contrast, the hallmark of CSA, of which there are various forms such as Cheyne-Stokes respiration, is cessation of airflow without respiratory effort. There are well described pathological mechanisms and clinical overlap between the two conditions. Pathophysiological underpinnings of OSA relevant to the development of atrial arrhythmias include hypoxia and hypercapnia, generation of negative intrathoracic pressure (Mueller manoeuvre) and multiple arousals from sleep – all these mechanisms may themselves effects atrial electro-mechanics as well as lead to activation of the sympathetic nervous system.

Focusing on OSA, there are several mechanisms responsible for the changes in autonomic nervous system (ANS) activity seen as a result of recurrent respiratory events. AF induction has been shown to be
increased by both vagal and sympathetic mediated mechanisms. Indeed, heart rate variability studies have shown either vagal or sympathetic dependent mechanisms prior to the onset of AF. 

Animal models with shutting of ventilation on end-expiration have shortening of ERP and neural firing from the monitored ganglionated plexi. Linz et al., further demonstrates the importance of the vagal stimulus, by demonstrating that negative tracheal pressure can lead to shortening of ERP which may be blocked by potassium channel blockade.

Animal experiments demonstrating vagal discharges in the absence of lung or chest wall motion have pointed to centrally mediated ANS effects. In human studies on lung transplant recipients, where there is absence of intrathoracic, intravascular receptors, or lung-inflation stimulated vagal afferents, observations of skeletal muscle sympathetic nerve activity (MSNA) have suggested independence from these mechanisms, i.e. a centrally mediated pathway. The ‘diving reflex’, another centrally mediated reflex, acts in opposition when triggered in apnoea by stimulating vagal efferent outflow resulting in bradycardia. In the presence of these afferents, however, lung inflation, via pulmonary stretch receptors, and the pulmonary vagal nerve, causes a reduction in sympathetic neural activity. Consequently, the reduction in stretch due to apnoeas in OSA patients may result in withdrawal of this sympathoinhibitory influence, and thus, a greater sympathetic outflow, particularly in the presence of hypoxia.

The intrathoracic pressure changes, as simulated by the Mueller manoeuvre, which are present in OSA, have been shown to have a
significant effect on cardiac haemodynamics by decreasing LA volume and increasing LV end-systolic volume culminating in a reduction in stroke volume. During the manoeuvre, as demonstrated by Somers et al., sympathetic activity is initially inhibited, perhaps due to the effect of stretch on the aortic baroreceptors opposing hypotensive stimulation of carotid baroreceptors. Adaptation of these mechanisms may lead to an increase in sympathetic activity however, and this may be augmented in hypoxia and hypercapnia. With release, sympathetic inhibition ensues resulting from due the effects of lung inflation, normalisation of oxygen and carbon dioxide levels, and a surge in blood pressure causing baroreceptor mediated sympathetic inhibition. On the other hand, end expiratory apnoea (i.e. without the Mueller manoeuvre) results in more sustained elevation in sympathetic activity. Adding complexity is baroreflex downregulation is seen with OSA and this may contribute to the chronically increased sympathetic tone seen in patients with OSA.

Carotid chemoreceptor outflow is augmented in response to acute and chronic intermittent hypoxia in patients with OSA. This results in sympathetic outflow along with increases in blood pressure and ventilation. Experimental acute hyperoxia (100% oxygen) decreases this hypersensitivity and results in a reduction in MSNA and blood pressure in OSA subjects. With chronic exposure to intermittent hypoxia, as demonstrated in animal models, there is a long-lasting increase in baseline carotid body sensory activity known as 'long-term facilitation' linked to reactive oxygen species which may be reversed with normoxia. This may partially explains the positive effects of CPAP
on sympathetic outflow. In addition to these two reflexes, the chronically elevated catecholamine levels seen with OSA may be contributed to by hypoxia induced catecholamine secretion from the adrenal medulla.

Also, in an ovine model of atrial fibrillation with blockade of the sympathetic nervous system, hypercapnia was found to cause a prolongation or atrial ERP and conduction times. Hypercapnia has been shown to be a potent stimulator to ventilation and sympathetic activity via the central chemoreceptors, more so than hypoxia, and even more so in the presence of hypoxia. Moreover hypercapnia can lead to a reduction of their inhibitory effect of pulmonary afferents, and thus a net increase in sympathetic tone. Hypercapnia has been shown to affect atrial electrophysiology in the absence of the SNS.

Few studies have examined acute changes in conduction velocity in the remodelled human atria, however, Oliveira et al. recently observed a prolongation of cardiac conduction times with carotid nerve stimulation in patients with sustained atrial fibrillation. We have demonstrated that there are dynamic changes linearly measured CT pointing towards a continually and significantly changing substrate. When challenged by a potential trigger such as a premature stimulus initiated by apnoea induced firing of ganglionated plexi, AF may follow, favoured by heterogeneity of electrical activity. These dynamic elements promoting AF are heterogeneously present throughout the night, and are additional to the chronic changes observed in the atrial myocardial substrate, described in a previous chapter in this thesis.
5.4.3 LIMITATIONS

There are several limitations to this study. This small cohort of patients was studied the night after undergoing an AF ablation. Acute changes to atrial electrophysiology may have occurred as a consequence of the ablation procedure. We did, however, avoid patients that had ablation in the RA, CS or inferior LA i.e. where substrate modification, linear ablation or ablation of complex fractionated electrograms may have affect proposed measurements. Furthermore, patients were given sedative and anaesthetic agents (midazolam and fentanyl). Whilst the effects of these agents were ‘reversed’ at the end of the procedure, the residual effects that the drugs may have had on their breathing characteristics, or transition of sleep stages at night, could not be accounted for. Patients were studied during sleep stage 2 for standardisation, as most of the patients did not exhibit deeper periods of sleep. This may have resulted from the procedure earlier in the day, or from discomfort and awareness of the experiment being performed at the time. The changes in NREM stage 2 may not reflect the quantity or quality of changes that may occur in other stages of sleep. Also, whilst we made every attempt to reduce the impact on the patient, the fact a 7F catheter was placed in the right jugular vein and cardiac pacing was occurring could have caused anxiety with its own sympathetic/parasympathetic (SNS/PNS) consequences. Catheter movement could not be evaluated, as fluoroscopy was not performed during the study in the coronary care unit. Data where catheter movement was suspected or interference was seen were, however, discarded.
The patients had different degrees of, and, components to their sleep disordered breathing – some patients exhibited CSA as well as OSA events. The degree of change in any given respiratory event maybe partly affected by the overall disease process in any given individual and this could not be accounted for in this model. Moreover, due to the small number of patients consenting for this study, we did not include any potential confounders such as the presence of disease modifiers (e.g. hypertension or atrial dilatation). We did however attempt to ‘standardise’ the measurements by using percentage change to quantify the differences (i.e. each event served as its own control and thus eliminated the potential for confounding subject characteristics that remained constant during the measurement period). We did not quantify the degree of sympathetic activation during the study. Arousals were recorded, however, given their distribution throughout the events, and the relatively small number of events examined, the design was not powered to demonstrate their potential impact and hence their presence was not included in the model. ‘Hangover’ effects from neighbouring events, preceding a particular event being measured could not be quantified. Latent effects recorded as “no change” may manifest seconds after the measurements were taken and may affect measurements in the following period, or many seconds after completion of measurements from that particular event, hence, evade detection. Linear conduction velocity is only a surrogate for cardiac conduction velocity, which, on a two-dimensional and three-dimensional level is much more complex. Small and perhaps still significant changes in ERP could not be detected beyond ±10ms. This was necessary in order to determine the ERP within
the time frame of events examined – to detect smaller changes in ERP would have necessitated several determinations of ERP in a given period, and this was not possible. Moreover, the experimental design and physical limitations due to catheter placement, and limited sleep time in stage II when measurements could be taken, allowed only one atrial site (proximal CS) to be analysed, thus precluding analysis of ERP heterogeneity throughout the atria. Ganglia function or the effects of ablation on ganglionated plexi were not evaluated and may have impacted on the study results.

5.5 CONCLUSION

In conclusion, HY and OA, probably via multiple mechanisms, cause acute electrophysiological remodeling of the atrial myocardial electrical properties. These dynamic changes are additional to the chronic changes observed in previous experiments, and may explain a propensity to nocturnal AF, and thus, contribute to the overall burden of AF seen in these patients.
Table 5.1
Baseline demographic data and measurements

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>7</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59±5</td>
</tr>
<tr>
<td>Male</td>
<td>5</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>30.5±1.8</td>
</tr>
<tr>
<td>Neck circumference (cm)</td>
<td>41.8±1.7</td>
</tr>
<tr>
<td>Hypertension</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medication usage</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin</td>
<td>7</td>
</tr>
<tr>
<td>Beta blockers</td>
<td>2</td>
</tr>
<tr>
<td>ACEI/ARBs</td>
<td>4</td>
</tr>
<tr>
<td>Class I anti-arrhythmics</td>
<td>1</td>
</tr>
<tr>
<td>Class III anti-arrhythmics</td>
<td>4</td>
</tr>
</tbody>
</table>

| LV systolic function            | 70±6 % |
| LA size (2d ECHO)               | 29.7±2.8 |
| LV posterior wall thickness     | 1.1±0.2 |
| Normal LV function              | 7     |

<table>
<thead>
<tr>
<th>Polysomnography study statistics</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>AHI (events/hour)</td>
<td>45±22</td>
</tr>
<tr>
<td>CAHI (events/hour)</td>
<td>10±18</td>
</tr>
<tr>
<td>SLEEP EFFICIENCY (%)</td>
<td>64±23</td>
</tr>
<tr>
<td>% NREM SLEEP</td>
<td>88±5</td>
</tr>
<tr>
<td>No of SaO₂ desaturation ≥ 3%</td>
<td>30±19</td>
</tr>
<tr>
<td>NREM SaO₂ - average</td>
<td>92±1</td>
</tr>
<tr>
<td>NREM SaO₂ - minimum</td>
<td>84±4</td>
</tr>
</tbody>
</table>

(BMI, body mass index; LV, left ventricular; LA, left atrial; AHI, apnoea-hypopnoea index; CAHI, central apnoea-hypopnoea index; NREM, non-rapid eye movement; Sa, saturation)
### Table 5.2

**Absolute change in (Δ) ERP, CT and CD between study periods compared to changes in reference events**

<table>
<thead>
<tr>
<th>REF EVENTS</th>
<th>ΔERP (%)</th>
<th>p</th>
<th>ΔCTCS5S1 (%)</th>
<th>p</th>
<th>ΔCTLAT-S1 (%)</th>
<th>p</th>
<th>ΔCTCS52 (%)</th>
<th>p</th>
<th>ΔCTLAT-S2 (%)</th>
<th>p</th>
<th>ΔCDCS (%)</th>
<th>p</th>
<th>ΔCDSL (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HY EVENTS</td>
<td>2.9 ± 2.3</td>
<td>&lt;0.001</td>
<td>5.0 ± 4.1</td>
<td>0.3</td>
<td>7.1 ± 8.0</td>
<td>&lt;0.001</td>
<td>12.4 ± 14.8</td>
<td>&lt;0.001</td>
<td>8.2 ± 8.5</td>
<td>&lt;0.001</td>
<td>17.6 ± 16.9</td>
<td>0.005</td>
<td>16.3 ± 13.4</td>
<td>0.002</td>
</tr>
<tr>
<td>HY EVENTS</td>
<td>6.0 ± 5.5</td>
<td>&lt;0.001</td>
<td>16.6 ± 8.4</td>
<td>&lt;0.001</td>
<td>10.2 ± 7.1</td>
<td>&lt;0.001</td>
<td>14.7 ± 12.4</td>
<td>&lt;0.001</td>
<td>7.3 ± 6.0</td>
<td>0.002</td>
<td>38.8 ± 33.5</td>
<td>&lt;0.001</td>
<td>50.6 ± 33.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HY EVENTS</td>
<td>4.2 ± 2.7</td>
<td>0.002</td>
<td>8.2 ± 6.3</td>
<td>0.002</td>
<td>6.7 ± 3.7</td>
<td>&lt;0.001</td>
<td>11.4 ± 8.0</td>
<td>&lt;0.001</td>
<td>5.4 ± 3.1</td>
<td>&lt;0.001</td>
<td>23.0 ± 17.0</td>
<td>&lt;0.001</td>
<td>18.2 ± 11.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HY EVENTS</td>
<td>2.5 ± 2.5</td>
<td>&lt;0.001</td>
<td>6.3 ± 4.9</td>
<td>0.001</td>
<td>5.4 ± 7.6</td>
<td>0.03</td>
<td>9.1 ± 7.8</td>
<td>&lt;0.001</td>
<td>4.5 ± 3.3</td>
<td>&lt;0.001</td>
<td>14.8 ± 10.9</td>
<td>0.029</td>
<td>19.4 ± 15.4</td>
<td>0.002</td>
</tr>
<tr>
<td>HY EVENTS</td>
<td>3.6 ± 6.5</td>
<td>0.004</td>
<td>12.1 ± 11.9</td>
<td>&lt;0.001</td>
<td>5.5 ± 4.4</td>
<td>0.002</td>
<td>10.2 ± 12.3</td>
<td>&lt;0.001</td>
<td>4.7 ± 4.2</td>
<td>&lt;0.001</td>
<td>28.6 ± 24.9</td>
<td>0.01</td>
<td>31.4 ± 21.6</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Note:**
- REF: reference
- HY: hypopnoea
- OA: obstructive apnoea
- ERP: effective refractory period
- CT: conduction time
- CD: conduction delay
A 20 polar (5-2-5-60-5-2-5mm spacing, St Jude Medical, St Paul, Minnesota, USA) diagnostic catheter was placed via a right jugular 7F sheath. It was positioned so that the distal group of bipoles sat securely in the coronary sinus, with the proximal pair of bipoles from that group sitting at the coronary sinus os. The proximal ten bipoles were thus positioned to record lateral right atrial electrograms.
Figure 5.2
Changes in ERP

This depicts changes in ERP for HY and OA events relative to period 1.

(HY, hypopnoea; OA obstructive apnoea; ERP, effective refractory period)
This depicts changes to conduction parameters for HY events relative to period 1.

(HY, hypopnoea; OA, obstructive apnoea; CS, coronary sinus; LAT, lateral RA; ERP, effective refractory period; CT, conduction time; CD, conduction delay)
Figure 5.4
Changes in CD and CT for OA events

This depicts changes conduction parameters for OA events relative to period 1.
(CS, coronary sinus; LAT, lateral RA; ERP, effective refractory period; CT, conduction
time; CD, conduction delay)
Figure 5.5
Percentages and directionality of significant changes in ERP*, CT# and CD#

HY EVENTS

EVENTS

The 2 panels display the percentage of events that had a significant positive, negative or neutral change for transition from period 1 to 2 (period_{1,2}), and 2 to 3 (period_{2,3}).

* A significant change in the ERP was considered to be ≥±10ms.

# A significant change in CT or CD was considered to be ≥±5 ms.

(OA, obstructive apnoea; HY, hypopnoea; CS, coronary sinus; LAT, lateral RA; ERP, effective refractory period; CT, conduction time; CD, conduction delay)
THE ACUTE EFFECTS OF HYPOXIA AND HYPERCAPNIA ON ATRIAL ELECTROPHYSIOLOGY: AN EX-VIVO RABBIT MODEL

6.1 INTRODUCTION

There is now mounting evidence demonstrating the relationship between obstructive sleep apnoea (OSA) and atrial fibrillation (AF).\textsuperscript{21,786,789} Recently, Stevenson et al., in an ovine open-chest model of sleep apnoea, suggested that hypercapnia, but not hypoxia, caused a differential recovery in effective refractory period (ERP) (which recovered quickly) and conduction time (which had a delayed recovery). These changes might lead to a substrate for AF in OSA and other respiratory disorders.\textsuperscript{200} Other studies have produced divergent results in hypoxia-induced electrophysiological changes in the atria.\textsuperscript{199,868,869} Lammers et al. has previously demonstrated, in isolated superfused left atria (LA), that the LA is made vulnerable to arrhythmia through wavelength shortening and an increase in inhomogeneity.\textsuperscript{198,199} They did not examine for reversibility of these acute changes with resolution of hypoxia.\textsuperscript{199}

We postulate that ERP and conduction are affected by both hypoxia and hypercapnia, and, that changes in these attributes may form a substrate for atrial fibrillation.
6.2 METHODS

6.2.1 SPECIMEN PREPARATION

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

Twenty-one New Zealand white rabbits (mean weight 2.4±0.3kg; aged 16±2wks) were given intravenous phenobarbital (30mg/kg) and heparin (1000 units/kg). A mid-sternal thoracotomy was performed and the heart, with attached lung tissue, was quickly excised and placed in ice-cold solution tissue bath of pH 7.4 containing in mM (NaCl 130, KCl 5.6, NaHCO$_3$ 24.2, CaCl$_2$ 2.2, MgCl$_2$ 0.6, NaH$_2$PO$_4$ 1.2, glucose 12). The LA was carefully excised and the pulmonary veins removed. The tissue was immediately transferred to the microelectrode array (MEA; Nucleus Medical, Adelaide, Australia) for immediate study.

6.2.2 EXPERIMENTAL DESIGN

The MEA setup is demonstrated in figure 6.1. The recording plate consisted of silver-wire electrodes (0.3mm x 0.1 mm) in a 9 x 9 format arranged at a 1.5mm pitch. This yielded 72 bipolar electrograms for electrophysiological recording. The MEA was housed in a sealed acrylic dish. The electrodes were wired to two high density circular connectors (MIL-D-38999, Amphenol Corporation, Wallingford, CT, USA) which
were connected to a computerised electrophysiology recording system (LabSystem Pro, Bard Electrophysiology, Lowell, MA, USA). Electrograms were sampled at 2kHz and filtered from 10-500Hz. The pacing stimulus (current: 0.1mA, pulse width: 0.5ms) was delivered by a cardiac stimulator (Micropace EPS cardiac stimulator, Micropace Pty Ltd, Canterbury, NSW, Australia).

The LA tissue was carefully placed over the MEA in the same craniocaudal and mediolateral orientation as in vivo with the epicardial surface in contact with the underlying electrodes. A lightweight mesh was placed over the tissue to ensure constant tissue contact with the underlying electrodes. A tightly fitting acrylic housing lid was prefabricated to allow tubing for gas mixture, and probes for oxygen (measured in parts per million) and pH monitoring (EAI 603 dissolved Oxygen probe, EAI pH2011 pH electrode, EA Instruments Ltd, London UK). The superfusate, in an enclosed beaker, was bubbled with experimental gas mixtures and an identical gas was allowed to fill the space between the MEA and the housing lid. In the baseline state the tissue was superfused with 95%O₂/5%CO₂ to maintain a pH 7.35-7.4. 1mM 2,3 butanedione monoxime was added to suppress contraction atrial artefacts. The superfusate was circulated in an open loop using a peristaltic pump (Minipuls 3, Gilson Inc., Middleton, WI, USA) and temperature was maintained at 37.5°C using a heater.

The experiment was designed in 3 stages (stage 1, baseline; stage 2, experimental; stage 3, recovery) each of 10 minutes duration, during which a full stimulation protocol (see below) was collected within two 5 minute periods (figure 6.2). Baseline recordings were performed in
stage 1 for all 21 left atrial specimens with ‘normoxia’ as the gaseous mixture (pH 7.35-7.4, 95%O$_2$/5%CO$_2$). During stage 2, the superfusate was immediately drained from the acrylic dish and replaced by superfusate bubbled to achieve one of 3 states: “moderate hypoxia” (pH 7.35-7.4, 21%O$_2$/5%CO$_2$/74%N$_2$; 43.5 ± 2.2% of the baseline oxygen content), “severe hypoxia” (pH 7.35-7.4, 5%CO$_2$/95%N$_2$; 16.22 ± 1.78% of the baseline oxygen content) or “hypercapnia” (pH 7.1-7.2, 10%CO$_2$/90%O$_2$). During Stage 3, the experimental superfusate was drained and replaced by superfusate (95%O$_2$/5%CO$_2$) to obtain the baseline state.

6.2.3 MODEL VALIDATION

To validate the model prior to commencement, the three stage stimulation protocol was carried out on 6 rabbit left atrial specimens using sham exchanges for stages 2 and 3 – i.e. experimental gas was 95%O$_2$/5%CO$_2$. These experiments demonstrated the viability of the atrial tissue over the 30 minute duration of the experiment without significant change in the atrial electrophysiological characteristics (figures 6.3 & 6.4).

6.2.4 ELECTROPHYSIOLOGY STIMULATION PROTOCOL

In each 5 minute time point, pacing was performed from two opposing corners of the MEA (one septal cranial, one septal caudal).

Tissue ERP was assessed via an eight beat stimulus drive train (S1; pacing cycle length (PCL) of 300ms, 200ms, 100ms) at twice diastolic threshold followed by a coupled extrastimulus (S2) introduced in
10ms decrements. ERP was defined as the longest S1-S2 interval not resulting in a propagated response. Each measurement was repeated twice and the total averaged. If the ERP differed by more than 10ms, a third determination of ERP was performed and the average of all the measurements recorded.

Tissue conduction velocity (CV) was assessed during a stable drive train (S1, PCL 300ms) and from the shortest propagated response S2 (functional conduction). Activation maps were created using previously validated semi-automated customised software with manual verification of each recorded electrogram and relative timing compared to the stimulus point. Local CV was assessed from vectors within each triangle of electrodes, and for each specimen an average CV was calculated. For all neighbouring recording points, the local phase differences in activation times were recorded, and for each quadruplet of electrodes, the largest value recorded. These were used to create phase maps and histograms as previously described. From these, conduction heterogeneity index (CHI) was derived from dividing the absolute conduction phase delay by the median conduction phase delay \( \frac{(P_{0.05}-P_{0.95})}{P_{0.50}} \).

6.2.5 STATISTICAL ANALYSIS

All continuous variables are reported as mean±SD. A mixed effects model was constructed to compare electrophysiological changes over the 3 stages. Experimental stage (baseline, experimental, recovery), pacing site, and their interaction, were modelled as fixed
6.3 RESULTS

6.3.1 MODEL VALIDATION

Whilst mean ERP, CV and CHI varied slightly over the 30 minute period (figure 6.3 and 6.4), mixed model analysis did not demonstrate a significant effect of stage 2 or 3 compared to stage 1 (ERP p=0.1, mean CV p=0.36, CHI p=0.8). There was no significant interaction between site of stimulation and stage (site*stage: ERP p=0.35; CV p=0.1; CHI p=0.9). Over the three cycle lengths tested, physiological rate adaptation remained intact (p< 0.001).

6.3.2 ATRIAL REFRACTORINESS

There were significant changes in ERP from baseline through the experimental period and into the recovery stage noted for all 3 experimental models at all 3 PCL examined compared to control specimens (all p<0.01). Mean changes in ERP, and ERP expressed as a percentage of ERP in stage 1 are presented in figure 6.3. In the mixed effects model, the changes in ERP were homogenous over the two sites (p=0.7).

With institution of moderate hypoxia, there was a mean increase in ERP to 124±25% of stage 1 (p<0.001). In recovery, there was a reduction in ERP, however, the final ERP remained elevated at
111±11% of baseline ERP (p=0.04). Physiological rate adaptation remained intact for the difference between a PCL of 100ms and higher cycles lengths (p<0.001), however, there was an interaction between stage and PCL suggesting an effect of moderate hypoxia on physiological rate adaptation (p=0.02), which was lost between PCL 200 and 300ms in stage 2 and 3.

In the LA specimens subjected to severe hypoxia, there was once again a marked effect on ERP (p<0.001) throughout the 3 stages. The ERP during this experimental stage demonstrated a mean increase of 136±17% (p<0.001) over baseline levels. As with moderate hypoxia, there was a small reduction in the ERP in recovery, however, the final ERP measurements still represented 118±26% (p<0.001) of the baseline mean. Once again, physiological rate adaptation was lost between the higher pacing cycle lengths in stages 2 and 3 (p=0.4).

When degree of hypoxia (moderate vs. severe) and experimental stage were modelled as the fixed effects, there was a more significant change in ERP noted with worsening hypoxia (p<0.001). There was no significant interaction for stage*degree of hypoxia at PCL 300ms & 200ms. However, the interaction term was p=0.04 at PCL=100ms resulting from the loss of capture seen as ERP rose above the PCL during severe hypoxia (and this was given a maximum value of 100ms for the model, thus blunting the observed change).

In the LA specimens exposed to hypercapnia, there was once again a marked effect on ERP (p<0.001), however, the pattern of these changes was different to that observed with hypoxia. Initially, during stage 2, there was a small change in mean ERP to 105±21% of baseline
Greater changes appeared to develop in the later stage of hypercapnia and in the recovery stage, where the mean change was $119\pm12\% \ (p<0.001)$ of baseline. In a similar fashion to that seen with hypoxia, there was loss of physiological rate adaption with hypercapnia ($p=0.4$).

6.3.3 ATRIAL TISSUE CONDUCTION VELOCITY AND HETEROGENEITY

There were significant changes in CV and CHI from baseline, through the experimental stage and into the recovery for all 3 experimental models. Mean changes in CV and CHI, and measurements expressed as a percentage of stage 1 values are presented in figure 6.4. These effects were observed regardless of the pacing site, i.e. they were homogeneous ($p=0.3$).

For moderate hypoxia, there were significant changes in both CV and CHI throughout the 3 stages compared to control specimens (CV and CHI S1, $p<0.001$; S2 $p<0.001$), irrespective of pacing site. In stage 2, there was a mean decrease in mean CV compared to baseline velocity (S1-CV $67\pm11\% \ (p<0.001)$; S2-CV, $78\pm13\% \ (p<0.001)$). With restoration of oxygenation, there was a modest improvement in mean conduction velocity compared to stage 2, however, these did not reach baseline levels (S1-CV 73$\pm5\%$, $p<0.001$; S2-CV 88$\pm14\%$, $p=0.02$) i.e. slowing of conduction persisted into recovery. This was also matched by CHI, which increased compared to stage 1 (S1-CHI 166$\pm21\%$, $p<0.001$; S2-CHI 144$\pm19\%$, $p<0.001$). As with CV, there was only mild, non-statistically significant improvement into the recovery stage with mean...
CHI remaining high in stage 3 compared to stage 1 (S1-CHI 165±16%, p<0.001; S2-CHI 129±12%, p=0.002).

With severe hypoxia, the overall changes observed in CV and CHI were also significant compared to the control specimens (CV and CHI S1, p<0.001; S2 p<0.001), irrespective of pacing site. In stage 2, CV was significantly lowered compared to stage 1 (S1-CV 52±7%, p<0.001; S2-CV 64±9%, p<0.001). With recovery, there was mild, non-statistically significant improvement in S1-CV as it remained low compared to baseline (S1 58±6, p<0.001; S2 75±15%, p=0.001). Similarly, there was a significant rise in CHI with severe hypoxia (S1-CHI 203±22%, p<0.001; S2-CHI 166±15%, p<0.001) and a significant residual increase in stage 3 (S1-CHI 189±22%, p<0.001; S2-CHI 145±13% p<0.001). With both moderate and severe hypoxia, marked areas of conduction slowing/conduction block were seen particularly in the S2 conducted beat (figure 6.5).

Comparing moderate to severe hypoxia, there was a greater degree of change with more severe hypoxia in S1-CV (p=0.01) and less recovery compared to moderate hypoxia (p=0.001). For S2-CV, whilst the changes with severe hypoxia were slightly greater (p=0.04) there was no statistical difference in overall recovery compared to the baseline state (p=0.1). For S1-CHI, there was a greater change with more severe hypoxia (p=0.006) and less recovery (p=0.02), and, similarly for S2-CHI (p=0.01 and p=0.03 respectively).

For hypercapnia, again, there was a significant change for CV and CHI compared to reference atria (CV and CHI, S1, p<0.001 S2, p<0.001) irrespective of the pacing site. However the pattern of conduction change
throughout the experiment was different for that observed with hypoxia. For CV, there was a decline with stage 2 (S1-CV 63±11%, p<0.001, S2-CV 58±10%, P<0.001). In stage 3, however, there was continual decline in CV when compared to baseline measurements (S1-CV 49±8%, p<0.001; S2-CV 56±5%, p<0.001). Furthermore, CHI increased with introduction of hypercapnia (S1-CHI 164±23%, p<0.001; S2-CHI 142±8%, p<0.001) and there was a continual increase in CHI into stage 3 (S1-CHI: 233±38%%, p<0.001; S2-CHI: 160±12%, p<0.001). Again, areas of conduction slowing/conduction block were seen, particularly in the S2 conducted beat.

6.4 DISCUSSION

6.4.1 MAJOR FINDINGS

This study utilised a MEA to characterise LA electrophysiological changes that occur during hypoxia and hypercapnia.

The changes seen are characterised by:

- Dose dependent increase in ERP with hypoxia, but a delayed increase with hypercapnia. There was only partial recovery of ERP with reversal to baseline conditions.
- Dose dependent reduction in mean CV for hypoxia with incomplete recovery in stage 3. With hypercapnia, the slowing of conduction progressed well into the recovery phase.
- Dose dependent increase in CHI with some resolution of these changes in the recovery phase from hypoxia. With
hypercapnia, there was continual increase of CHI into the recovery stage.

- Areas of marked conduction slowing/conduction block during hypoxia and hypercapnia without resolution in the recovery stage.

Together, these findings suggest that hypoxia and hypercapnia have a marked, but different acute effect on the atrial myocardium, and the changes in ERP, CV and CHI may contribute to a myocardial substrate capable of allowing perpetuation of atrial fibrillation.

6.4.2 COMMENT

In 1983, Guilleminault et al. reported the association and prevalence of atrial arrhythmias in patients with OSA and particularly, their nocturnal prevalence compared to the general community.\textsuperscript{19} Larger retrospective cohorts, such as those from Olmstead County over a 20 year period, have provided further evidence for this relationship demonstrating a 14\% cumulative probability of developing AF during a 15 year follow-up in patients with OSA.\textsuperscript{21} Various measures of OSA severity were also found to be relevant and most interestingly the magnitude of nocturnal oxygen desaturation (adjusted HR 3.29). Ventricular arrhythmias and indeed, sudden cardiac death have also been associated with OSA.\textsuperscript{782,871,872} ‘The Sleep Heart Health Study’, a multicentre longitudinal study investigating the cardiovascular morbidity associated with sleep disordered breathing, found a higher prevalence of AF in patients with an AHI \( \geq 30 \). From this cohort, sleep studies were
analysed, and concluded that there was an 18-fold increase in the relative risk of nocturnal arrhythmias within 90 seconds of a respiratory event in patients with obstructive sleep apnoea.784,788

In the literature, the effect of hypoxia on cardiac conduction has been variably reported. In 1925, Resnik published his observations on the effect of anoxaemia on the canine heart with reference to its relationship to auricular fibrillation.873 During his experiments, he found that the atrial ERP first decreased, then increased, causing various degrees of ‘intra-auricular block’. With return of normal oxygenation, refractory periods returned to figures above those originally seen. The effect of anoxia on the action potential of isolated rabbit sinoatrial nodes was studied by Kohlhardt et al. demonstrating sinoatrial cell depression brought about by changes to threshold potential up-stroke velocity and overshoot of the slow inward current.874 In further studies from the 1970’s, hypoxia was found to decrease the action potential amplitude in the sinoatrial node and the atrioventricular node, but the effect was not seen in atrial tissue. There was however, inhomogeneity of atrioventricular nodal conduction with hypoxia.868 Rabbit atrial conduction was studied by Lammers et al. however, their experiment differed in many ways to ours.199 Hypoxia was produced using an \( \text{N}_2 \) gas mixture. The tissue remained this way for 60 minutes. With the onset of hypoxia, there was an increase in the ERP with a concomitant decrease in the action potential duration. With maintenance of hypoxia, after this initial increase in ERP, a shortening of refractoriness was observed with some values less than control. Furthermore, there was a marked increase in the CHI both in basic rhythm and premature beats. In contrast, recent
observations from Stevenson et al.’s ovine model, where hypoxia was induced after exclusion of the sympathetic nervous system, demonstrated no effect on the electrophysiological parameters recorded during hypoxia. Interestingly, Klause et al. noted that changes produced by hypoxia, such as reduction in the ERP, were markedly decreased by vagotomy. Our findings from this experiment are similar to those of Lammers et al. We have also demonstrated that, with a return to baseline oxygen levels, there is a shortening of ERP. However, these did not return to baseline levels during the 10 minute recovery stage.

Webb et al., in studies demonstrating the effect of varying degrees of hypoxia on atrial contractility, found slowing of conduction with anoxia and recovery, even after complete contractile failure of the tissue, with restoration of oxygen levels. Lammers et al. demonstrated an acute reduction in CV within 10 minutes of induction of hypoxia CV, followed by some mild improvement over the subsequent hour. These changes were attributed to inactivation of fast sodium current and cardiac cell uncoupling. A greater degree of conduction inhomogeneity was seen with worsening hypoxia, indicating hypoxia induced electrical uncoupling or differences in generated membrane current as previously postulated. Similar to our study, areas of conduction block were seen and associated with marked increase in the CHI. Conduction heterogeneity was higher in the prematurely conducted beats. Our study further suggests that these changes are partially reversible after hypoxia recovers, although our experiment was unable to determine the time frame over which CV and CHI may fully recover (see
limitations). These conduction defects suggest that the heterogenous effects of hypoxia on different fibres causes heterogeneous amplification of anisotropy as has previously been described in isolated ventricular muscle. In summary, our study shows a dose dependent effect of hypoxia on the atrial myocardium ERP, CV and CHI and, the disparate observations of other investigators are perhaps dependent on the experimental models utilised.

The data published recently by Stevenson et al. are the first to clearly describe the changes in refractoriness under conditions of carbon dioxide excess. In this open-chest ovine model, with increasing end-tidal CO₂ there was a linear increase in ERP that remained prolonged compared to baseline during the maintenance period. There was a reduction to baseline ERP with restoration of normal CO₂ gas tension. Conduction times were also increased in the right atrial specimens, and, despite approaching baseline measurements during withdrawal of hypercapnia, remained significantly elevated. Similarly, functional conduction delay during S₂ extrastimulus testing demonstrated decreased CV. Other earlier studies on electrical coupling between cells, such as that measuring conduction in Xenopus embryos, observed found that reducing extracellular pH caused an increase in junctional resistance between the cells resulting in their electrical uncoupling, culminating in reversible abolition of current flow. They proposed the effects of CO₂, rather than the effects of intracellular calcium, were primarily responsible for the cellular uncoupling as was also shown by White et al. examining isolated rat ventricular junctional resistance during CO₂. Potential mechanisms of change were described previously by
Vorperian et al. showing hypercapnic acidaemia selectively and reversibly slowed ventricular conduction in a dog model, and, more recently, that pkA-induced phosphorylation of Cx43 is suppressed by hypercapnic acidosis and reversed by cAMP augmentation of connexin phosphorylation. In our experiment, ERP continued to rise into the recovery period, demonstrating a delayed effect with slower reversal, although, the extent and time frame of reversal could not be fully evaluated in this model. The CO₂ change brought about in the experiments of Stevenson et al. were over a one hour period which may have allowed a gradual recovery of the atrial tissue and restoration of refractoriness, however, despite this slow return to baseline CO₂ levels, there was persistent reduction in conduction velocity at completion of their experiment.

6.4.3 LIMITATIONS

The limitations of this study are several. Firstly, the oxygen and pH levels seen may not reflect the changes in oxygen and CO₂ tensions seen in humans with OSA or other respiratory diseases. Furthermore, the time frame over which these changes occur in humans is different – the time spent in each stage during this experiment was necessary to collect the data using standard electrophysiological techniques. Isolated tissue preparations may not model real life myocardial changes – in particular, the other stressors applied to the myocardium during episodes of OSA such as sympathetic drive, vagal tone, intrathoracic pressure changes etc are not included in this simplified model. Tissue fibre orientation was not examined or controlled for apart from maintaining
orientation of the atrial tissue on the MEA, which may be particularly important when referring to changes in tissue anisotropy, inhomogeneous conduction and conduction block. A ‘stunning’ effect on the myocardium from the rapid change of gaseous milieu in this experiment cannot be ruled out and it is not known what may happen in the long term if these experimental conditions are maintained. Our own investigations in creating the experimental model demonstrated that the tissue did not survive beyond 30 minutes with substantial changes in electrophysiology and eventual non-response from the tissue. A model lasting 1 hour was previously described by Lammers et al\textsuperscript{199} and in these experiments, there was a rise in the ERP within the first 10 minutes, however, despite continuation of hypoxia, the ERP then began to return to normal or lower than normal values. Whilst our experiment showed similar changes in ERP during 10 minutes of hypoxia, it is possible that the ERP could start to decrease back to baseline measurements. Further to this, due to the duration of the recovery period, we could not evaluate the timeframe to a full recovery of baseline electrophysiology, and this limits our interpretation of data in this stage.

We did not attempt to induce AF during the different stages, as induction would have resulted in loss of the specimen due to inability to reverse the arrhythmia within the specified time frame. Furthermore, potential acute electrical remodeling due to AF would potentially interfere with ongoing electrophysiological evaluation in subsequent stages.
6.5 CONCLUSION

We have demonstrated a direct acute remodeling effect of hypoxia and hypercapnia on the atrial myocardium. These changes are characterised by an increase in tissue refractoriness, a reduction in conduction velocity and an increase in conduction heterogeneity. These electrophysiological changes could occur to differing extents in a given individual depending on the degree and duration of hypoxia/hypercapnia, together with other potentially pro-arrhythmic stimuli associated with obstruction to breathing seen in OSA. These dynamic electrophysiologic changes provide some insights into the development of atrial arrhythmia in association with acute respiratory events.
The MEA was housed in a sealed acrylic dish with a prefabricated lid to allow for gas tubing, oxygen and pH monitoring. The recording plate consisted of silver-wire electrodes (0.3mm x 0.1 mm) a 9 x 9 format arranged at a 1.5mm pitch yielding 72 bipolar electrograms. The electrodes were wired to 2 high density circular connectors connected to a computerized electrophysiology recording system. A peristaltic pump allowed the superfusate to circulate in an open loop. Temperature was maintained at 37.5 °C.
The experiment consisted of three 10 minute stages. All left atrial specimens were examined in the baseline stage (normoxia, stage 1) followed by an experimental stage (stage 2) and a recovery stage (stage 3). In stage 2, the specimens were subjected to either ‘moderate hypoxia’, ‘severe hypoxia’, or, ‘hypercapnia’.

Figure 6.2
The experimental design
Figure 6.3
Changes to mean ERP in reference atria and experimental atria

Changes to mean ERP during baseline (stage 1), experimental stage (stage 2) and recovery (stage 3) for a) reference, b) moderate hypoxia, c) severe hypoxia and d) hypercapnia.  e) Percentage change of stage 1 ERP during moderate hypoxia, severe hypoxia and hypercapnia for PCL=300ms. (ERP, effective refractory period; PCL, pacing cycle length)
Figure 6.4
Changes to mean CV and CHI in reference atria and experimental atria

(a) Changes to mean S1 CV

(b) Changes to mean S2 CV

S1 CV as a percentage of stage 1

S2 CV as a percentage of stage 1

(a) Changes to mean S1 CHI

(b) Changes to mean S2 CHI

S1 CHI as a percentage of stage 1

S2 CHI as a percentage of stage 1

Figure legend overleaf
Figure legend 6.4

Changes to mean a) CV and b) CHI, calculated from the drive train (S1, PCL=300ms) and shortest propagated response (S2) during baseline (stage 1), experimental stage (stage 2; moderate hypoxia, severe hypoxia, hypercapnia) and recovery (stage 3). (CV, conduction velocity; CHI, conduction heterogeneity index; PCL, pacing cycle length)
Figure 6.5
Activation maps and corresponding phase histograms for S1 & S2 conducted beats

Figure legend overleaf
Figure legend 6.5

Representative activation maps created from a rabbit atrium subjected to severe hypoxia in stage 2. Plaque activation commences in the bottom left hand corner (red) and proceeds across the LA to the latest point of activation (purple). (LAT; Local activation time are presented (ms), but average conduction velocity was used in the analysis). Isochrones have been constructed at 2ms intervals. Isochronal crowding is seen during severe hypoxia and is indicative of slower conduction. This is more marked in the S2 conducted beat with demonstration of marked conduction slowing/conduction block. There was less slowing during recovery, but not to the baseline recordings. Corresponding phase histograms and conduction heterogeneity indexes (CHI) are also demonstrated. CHI during S1 and S2 were higher with severe hypoxia and again, there was some recovery, but not to baseline levels.
7 SUMMARY

This thesis has examined the relationship between AF and OSA in the context of patients presenting for invasive management of the arrhythmia with radiofrequency ablation. These studies have provided many important insights into the presentation of AF for ablation, the prevalence of OSA in this symptomatic arrhythmia population, AF symptoms scores, duration of the arrhythmia, and the impact OSA has on the atrial myocardium, nocturnal atrial electrophysiology, and arrhythmia free outcomes following ablation. This knowledge further expands our understanding of the acute and chronic remodeling caused by OSA, and provides an avenue for future studies directed at the treatment of OSA in the management of AF.

In chapter 2, we initially examined the differences in the atrial substrate during induced or spontaneous AF in a population of patients with paroxysmal and persistent AF. This analysis was carried out in the absence of knowing their sleep disordered breathing status. We compared the effects of arrhythmia duration (paroxysmal vs. persistent) on electrogram voltage and signal fragmentation. In patients with persistent AF, atria were larger, and electrograms had reduced amplitude and greater signal fragmentation. The degree of change in the atria was not homogeneous with some atrial segments demonstrating greater substrate changes than others. There was a clear relationship between a reduction in voltage and the presence of signal fragmentation characterising the changes in the underlying myocardium. There was
less chance of terminating AF immediately after PVI if the fragmentation index was high; however, longer term outcomes appeared more reliant on atrial size.

In chapter 3, the clinical features of patients with symptomatic atrial fibrillation presenting for consideration of ablation therapy were detailed. Importantly, from this study, was the finding of a high prevalence of OSA present in this population of patients who, as a group, did not score highly on the Epworth Sleepiness Scale. Over half of the patient population had at least moderate OSA. Moreover, there was a relationship between the severity of OSA and an increased symptomatic burden of arrhythmia at presentation. There was also a relationship between the persistence of AF and the severity of the OSA. This finding is particularly important as it suggests greater alteration of the underlying atrial myocardium capable of sustaining AF. Finally in this study, it was demonstrated that OSA affects the outcome from AF ablation with less patients remaining in sinus rhythm on follow-up. This is in keeping with data presented in a recent meta-analysis.\textsuperscript{791} It suggests that patients with OSA have a more negatively remodelled substrate, and this atrial alteration is less responsive to the effects of ablation. Also, given the multiple pathways discussed in this thesis capable of altering the myocardium, in the presence of ongoing OSA, this process will continue to adversely affect the atrial tissue possibly underdoing any potential positive outcome from radiofrequency ablation.

Given the findings of chapter 3, chapter 4 was designed to detail (for the first time to our knowledge) the electroanatomic changes that occur with untreated chronic OSA. This information allows a greater
understanding of the factors that predispose to maintenance of arrhythmia (and failure to achieve long term sinus rhythm). In the group of patients with moderate to severe untreated OSA, when examined remote from episodes of AF, a markedly altered electrical substrate was documented. This was characterised by prolonged conduction times along linearly placed catheters, site-specific conduction delay and prolongation of the surface ECG P-wave. Furthermore, there was prolongation of corrected sinus node recovery times, albeit, they remained within normal limits. There was no significant difference for ERP between the two groups in the baseline state; however, this was further examined in subsequent studies.

Further evidence of conduction slowing was seen in analysis of the electroanatomic maps created. This method has been previously used to determine underlying substrate changes in other disease states such as congestive cardiac failure and mitral stenosis. It was found that in OSA patients, there were bi-atrial structural abnormalities characterised by an increase in atrial size, extensive areas of low voltage signal and regions of electrical silence, which may represent loss of myocardium, fibrosis or underlying conduction dissociation. These changes appeared to be worse in the left atrium compared to the right. Furthermore, there were greater proportion of electrograms displaying features suggesting underlying abnormal conduction, collectively called complex electrograms and consisting of fractionated signals and double potentials.

Regional conduction slowing was also delineated from local activation timing maps created during collection of points. Together,
these findings indeed suggest a more diseased atrial substrate in patients with a worse degree of OSA. Given the findings of Verma et al.\textsuperscript{845} highlighting the importance of areas of atrial electrical silence in the long-term recurrence after pulmonary vein antrum isolation in AF, the remodeling seen in this study may partly explain the procedural failure documented in our previously described study and indeed, observed by many other investigators. This therefore suggests two important practice points – firstly that extensive atrial mapping for scar-related pro-arrhythmic isthmuses and areas that could contribute to the maintenance of AF may be necessary to improve procedural outcomes in patients with OSA, and secondly, that treatment with CPAP may be beneficial in protecting the myocardium from future adverse remodeling from the same ongoing process. Whether CPAP should be instituted in the post-operative period to prevent adverse remodeling from ongoing OSA, or beforehand for several months, to perhaps encourage positive remodeling that may reduce the chance of procedural failure, or indeed, reduce the need for an invasive strategy at all, is still not known.

Given OSA causes marked dynamic changes e.g. to transthoracic pressure, deoxygenation/reoxygenation, hypercapnia, autonomic nervous system modulation, and blood pressure, we sought to further determine the acute changes to atrial electrophysiology during periods of respiratory disturbance and subsequent hypoxia. This study was difficult for many reasons and had many limitations as outlined in the chapter, however, the results highlighted a dynamic and heterogeneous response to SDB events. Hypopnoeas and obstructive apnoeas were both associated with marked changes to single site ERP measurements at
the proximal coronary sinus, and changes in conduction times over the coronary sinus and lateral RA. There was also a marked change in localised conduction delay. In general, the ERP changes were more marked for obstructive events, as was the prolongation of conduction parameters. Given these changes were occurring in patients with severe OSA, and that it has been shown in a previous study that these patients display a greatly modified substrate, these findings of a dynamic electrical substrate are important in the pathophysiology of nocturnal triggering of AF. Indeed we did not document AF per se in this small group of patients (except for one patient as previously mentioned) because the protocol (as accepted by the institutional ethics committee) necessitated the overnight recordings be conducted in patients undergoing an ablative procedure for AF. In patients untreated with ablation, with undiagnosed OSA, the presence of dynamically changing atrial electrophysiology on a background of an adversely modified atrial myocardium is likely to allow triggering and perpetuation of AF. Hence, CPAP may not only allow positive remodeling over time, but may also work in the short term to reduce the dynamic changes occurring many times an hour in these patients.

In Chapter 6, an animal study was devised to evaluate directly the acute effects of gaseous change on the atrial myocardium. Rabbit atria, freshly dissected via a thoracotomy, were placed on a microelectrode array housed in a purpose built sealed acrylic dish. Various gas mixes were employed to simulate moderate hypoxia, severe hypoxia or hypercapnia. During the baseline stage, i.e. ‘normoxia’, stable state electrophysiological properties of the left atrial tissue were recorded. In
the second stage, the electrophysiological response to degrees of hypoxia or hypercapnia was examined. The noxious gaseous state was reversed back to baseline values in stage 3 and again atrial electrophysiology examined. The findings demonstrated a marked dose dependent effect of hypoxia with an increase in ERP, slowing of conduction and greater conduction heterogeneity, which only partially reversed with restoration of baseline. Hypercapnia caused a delayed effect with ERP continuing to rise into the recovery stage associated with increasing conduction slowing and heterogeneity. Importantly, areas of marked conduction slowing and conduction block were seen with the noxious stimulus and these did not recover fully. Together the observed changes seen with hypoxia and hypercapnia caused significant alterations in the myocardium to different degrees, and thus may be responsible for the observed relationship between sleep disordered breathing events and the nocturnal onset of atrial fibrillation. Furthermore, in the long term, it is plausible that these constant changes in atrial electrophysiology may lead to more chronic alterations in atrial electrophysiology additional to the other causes thought to be important such as activation of the sympathetic nervous system, oxidative stress, and inflammation.
8 FUTURE DIRECTIONS

This thesis has documented important aspects of the presentation and outcomes of AF in the presence of OSA, together with characterisation of chronic adverse remodeling, and dynamic changes in atrial electrophysiological properties secondary to episodes of nocturnal respiratory disturbance. This has provided important insights into the mechanisms of AF triggering and maintenance in patients with concomitant OSA.

One of the main findings from this study was the adverse remodeling that occurs to the atrium as a result of chronic untreated OSA. Clinical studies such as this, and others using similar methodology, examine the effects of a diverse range of processes that cause a reduction in signal voltage recorded at the end of an ablation catheter. From these observations, we assume the underlying myocardium is thinned or even scarred in the case of severe reduction of voltage below the recording system’s sensitivity. The precise mechanisms and the heterogeneity of changes that lead to these simple clinical observations are not clear.

The important data presented by Oakes et al.\textsuperscript{267} demonstrating the utility of DE-MRI for detection and quantification of atrial scar, and the use of this as a predictor of AF recurrence, could be extended to patients with OSA. Perhaps prior assessment of the atria may allow further risk stratification and identification of patients that have excessive scar burdens and are at increased risk of failing an invasive attempt at
maintaining sinus rhythm. Moreover, systolic and diastolic abnormalities in OSA patients when assessed by echocardiograph and cardiac MRI have been shown to reverse as early as 3 months with CPAP. Colish et al. also demonstrated a reduction in left atrial volume index with CPAP over 1 year. Further assessment with DE-MRI in patients with OSA and the effects of CPAP over time will lend further insights into the beneficial effect of this therapy on promoting cardiac remodeling and reduces overall burden of AF.

Clearly other studies have demonstrated some degree of reversibility after removal of the offending mechanism, e.g. valvuloplasty in mitral stenosis. Also our study uncovered potential sinus node remodeling, albeit the CSNRT’s were within acceptable limits. Bradycardia in the setting of OSA has been a longstanding observation made by many investigators. Moreover, AF has been shown to cause adverse remodeling to the sinus node, and indeed, an abnormal atrial substrate has been documented in patients with sinus node disease. How OSA in particular affects sinus node function in the long term is not clear, especially in the absence of documented AF.

There are data available, as discussed in the literature review, demonstrating the importance of CPAP therapy in combating the adverse outcomes of OSA. We have shown that OSA has a severe impact on the human atria causing adverse myocardial remodeling and dynamic changes in electrophysiology. Further studies are necessary to clarify the exact mechanisms leading to atrial myocardial changes, and importantly, whether they modifiable or reversible with CPAP. This raises that question, should treatment of this disease with CPAP (before
considering more invasive strategies such as pulmonary venous isolation) be the standard of care? More importantly is consideration of the possibility of OSA in the first place.

The patients in our studies were referred for ablation, but were not previously diagnosed with OSA. Furthermore, these patients did not have clear ‘OSA syndrome’ per se (daytime sleepiness was not a prominent symptom on presentation). Hence, the diagnosis should be considered in all individuals presenting for AF management.

Given the well established relationship between OSA and obesity, it should be strongly recommended that polysomnography be performed in all obese patients. However, given the fact that some of the patients in our study were not obese, consideration of the diagnosis of OSA should be made in all individuals. Perhaps home polysomnography, such as the Somté device, or even simpler devices measuring nocturnal oxygen saturations, should be considered. The ramifications of missing the diagnosis of OSA are chronic, and acute adverse remodeling that will likely lead to failure of invasive strategies and make medical therapy less successful. Further to this, OSA has an affect on other cardiac functions (including ventricular systolic contraction and diastolic compliance). This highlights the importance of CPAP in these patients as even an asymptomatic ‘stable’ patient with AF could become symptomatic over time if the arrhythmia exists in the company of a disease capable of adversely remodeling the ventricles.

Whether more extensive biatrial mapping allows for more adequate substrate modification and better outcomes, is currently not known. This is an important topic for future study – and equally as
important. Exactly what structures should be the target of ablation after the pulmonary veins are isolated? Given the importance of the ganglionated plexi demonstrated in other studies, and, the findings of a dynamic substrate in our study, no doubt reflecting in part autonomic changes, should these structures be a more focussed target in the ablative procedure? Perhaps more importantly, is the role for CPAP as a complimentary treatment to, or perhaps even a replacement for ablation in patients with AF and OSA?

Interesting works by other investigators have sought to further clarify the effects of the autonomic nervous system and its interaction with the ganglionated plexi in particular. Future work is warranted to more clearly characterise the effects of OSA on the autonomic nervous system with particular reference to the atria. Whilst other investigators have brought insights into the mechanisms of AF in OSA and determined that blocking specific channels may reduce AF inducibility in animal models, how this translates into the human atria is unclear.

Further to this, the end stage effects of untreated OSA on the sympathetic nervous system, and how this relates to hypertension in these individuals has been addressed in the literature. Whether these episodes related to the onset of AF has not been noted and would make for an interesting study given the availability of 24 hours ambulatory blood pressure and Holter monitors.

Assessment of AF post ablation often relies on the utility of repetitive use of Holter monitors, and assessment of symptoms. However, symptoms are not reliable in quantifying the presence of AF given the heterogeneity of presentation within and amongst individuals.
This study did not use an implantable loop recorders (ILR) to assess AF burden. Joshi et al. examined recurrences post PVI using ILRs and demonstrated that freedom of AF within the first 2 weeks following ablation significantly predicted long term AF freedom.\textsuperscript{881} This has also been demonstrated to be a useful modality for assessing AF burden after AF ablation during coronary artery bypass grafting.\textsuperscript{882} Perhaps use of ILRs before AF ablation in OSA patients may be an interesting study modality to relate atrial and ventricular electrical activity to daytime sympathetic activation and its effects, e.g. swings in blood pressure, and perhaps nocturnal modulation of the autonomic nervous system leading to AF. CPAP usage and its effects could be evaluated by monitoring AF episodes with an ILR prior to ablation.

There are currently studies underway, such as the SAVE study mentioned briefly in the literature review, that will offer important observations about the impact of CPAP on cardiovascular outcomes in patients with OSA and cardiovascular disease. Specific studies are warranted to determine if treatment of OSA before ablation offers an improvement in outcome from radiofrequency ablation, or indeed, delays, or even abolishes the need for an invasive approach.

This thesis has demonstrated OSA has important ramifications in the presentation, characterisation and ablation of AF, acutely and chronically. It remains to be shown whether treating this modifiable risk factor with CPAP has an impact on the burden of AF, the presentation for AF ablation and the arrhythmia free outcomes after ablation.
9

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