Thesis title:

Obesity and Atrial Electrical and Mechanical Remodeling: Implications for Atrial Fibrillation.

Candidate name:

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Academic institution and discipline:

The University of Adelaide, Discipline of Medicine.
Thesis by publication structure and acknowledgements

The following thesis is the product of 5 years of work investigating the relationship between obesity and atrial fibrillation mechanisms and therapy. It is presented as a thesis by publication. The thesis abstract provides an overview of the research questions addressed and the hypotheses examined and summarizes the methodologies results and conclusions. The chapter 1 is an introductory review of the pertinent literature, forming the background for the following experimental work. The summary of the introductory chapter and the concluding chapter 5 has been presented in the form of a review paper to the peer reviewed journal, Obesity Reviews (“Obesity and Atrial Fibrillation”, Article first published online: 24 JUL 2013 DOI: 10.1111/obr.12056).

Chapter 2 is the preclinical manuscript investigating the atrial structural, functional and electrical changes with progressive weight gain. The experiment utilizes cardiac magnetic resonance imaging, high density multi electrode epicardial electrophysiological measurements, histopathology and molecular analysis in an Ovine model of progressive weight gain. The paper was presented and awarded first prize at the Cardiac Society of Australia and New Zealand Ralph Reader Award 2011 basic sciences category and runner up at the Asia Pacific Heart rhythm society Young Investigator Award 2011. The manuscript has subsequently been published in the Heart Rhythm journal (“Obesity Results in Progressive Atrial Structural and electrical Remodeling: Implications for Atrial Fibrillation”, Volume 10, Issue 1, January 2013, Pages 90–100). Chapter 3 is a single centre randomized and controlled clinical study into the
impact of lifestyle intervention, focusing on weight and cardio metabolic risk factor management, on atrial fibrillation symptoms, arrhythmia frequency, arrhythmia duration and cardiac structure. The manuscript has been presented and awarded first prize in 2012 at the American Heart Association Samuel Levine Young Investigator Award and is currently in the external peer review domain for publication. Chapter 4 is a study into the role of weight loss on pericardial fat burden and its relationship to semi-quantitative atrial fibrillation burden. The clinical study is a sub-study of the above, utilizing cardiac magnetic resonance imaging to quantify pericardial fat and cardiac chamber volumes, anthropometry and serum biochemistry. The paper was presented and awarded first prize in 2013 at the American College of Cardiology Young Investigator Award clinical category presentations. The paper will be submitted for peer review and publication.

This thesis could not be completed without the guidance, mentorship, patience and support of my supervisor, Professor Gary Wittert. In addition, to the support and contribution of all co-authors on each manuscript, a special mention is made to my friend and colleague Dr Darryl Leong. His insight, dedication and expertise have been immensely invaluable to seeing through the completing of each manuscript chapter.
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Thesis declaration:

I certify that 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. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Dr Hany Abed
Abstract

Background

Epidemiological evidence identifies obesity as an independent risk factor for atrial fibrillation (AF). Additionally, therapeutic outcomes for AF appear to be adversely affected by the presence of obesity.

Conditions associated with AF such as hypertension, obstructive sleep apnea, coronary disease and cardiac failure have common salient atrial electro-structural features, predisposing to arrhythmias. Many of these conditions are also associated with obesity and atrial hypertension. However, the degree by which obesity itself, independent of confounding hemodynamic changes, results in atrial electro-structural changes favoring arrhythmogenesis remains unknown.

Aims

The aim of our first study was to determine, using an ovine model, the electro-structural changes resulting from weight gain and obesity, and the contribution of the accompanying hemodynamic abnormalities. Following characterization of the obesity related atrial “substrate”; we investigated, in humans with atrial fibrillation, whether weight loss with cardio-metabolic risk factor management reduces arrhythmia burden, disease severity and structural correlates of reverse remodeling.

Hypotheses:
(I) Progressive weight gain promotes pro-arrhythmic atrial changes. (ii) Weight reduction combined with effective management of obesity-related co morbidities has favorable effects on AF severity and burden. (iii) Weight reduction and risk factor management has a favorable effect on atrial remodeling and pericardial fat volume (PFV).

Methods

Atrial structural (cardiac MRI), histological (tissue infiltrates and pro-fibrotic mediators) and electrical (tissue conduction and excitability) changes accompanying progressive weight gain over 8 months through ad-libitum calorie-dense feeding, were determined in male sheep sampled at baseline, 4 and 8 months (10/group).

The clinical study was conducted as a single center randomized prospective trial, to investigate the effect of weight and cardio-metabolic risk factor management on AF severity, AF burden, atrial structure, myocardial mass and pericardial fat volume. The study utilized a physician-led weight and risk factor management program. This was compared to a parallel control group provided with brief lifestyle counseling and daily supplementation with marine triglycerides.

Results

The pre-clinical work showed that diet-induced obesity was accompanied by a progressive increase in atrial size, tissue inflammatory, lipid and fibrotic infiltrates. Molecular markers of pro-fibrotic mediators were also increased. There was slowing in
conduction velocity, heterogeneity of conduction dispersion and greater AF burden. The electrical abnormalities persisted following statistical adjustment for systemic and atrial hypertension and the changes were more profound with greater increase in weight.

The clinical work demonstrated an effective reduction in AF burden and severity, using a standardized validated AF severity questionnaire and ambulatory rhythm monitoring. In addition, there was a reduction in atrial size and ventricular wall thickness accompanying a favorable cardio-metabolic risk profile. There was a favorable reduction in PFV, height-indexed atrial volumes and myocardial mass. On post-hoc analysis PFV was predictive of the reduction in AF severity scores.

Conclusion

Diet induced obesity resulted in atrial conduction and structural abnormalities independent of systemic and left atrial hypertension, suggesting an obesity-specific effect.

Our translational work shows that the burden of AF may be reduced through effective weight loss and appropriate management of the underlying metabolic derangement. Moreover, pericardial fat volume is independently predictive of AF severity and this depot is amenable to lifestyle intervention.

Subsequent investigation requires further analysis of inflammatory markers, molecular pathways regulating fibrogenesis and myocardial electrical activity and the effect of
pharmacological inhibition of key mediators. Long-term outcome studies to determine maintenance of benefit are also required.
Chapter 1

Introduction

Obesity epidemic

The current prevalence of obesity and its future projected incidence has been recognized as a significant public health crisis challenging most developed nations and affecting a wide age range group \(^1\text{-}^3\).

Either in isolation or in conjunction with other co morbidities such as the metabolic syndrome (insulin resistance, hypertension and dyslipidemia) and obstructive sleep apnea (OSA), obesity and its associated conditions are a growing national epidemic, the full impact of which is slowly being realized. Recent data estimates that overweight and obesity affects 60% of the Australian adult population and up to a quarter of children and adolescents \(^4\). Moreover, the overall trend of overweight individuals appears to increase in parallel with age, reaching a plateau at 65-74 years of age, a group also at significant risk of atrial fibrillation \(^5\).

Atrial fibrillation epidemic

Atrial fibrillation (AF) has been described as the epidemic of the new millennium \(^6\). The prevalence is expected to continue increasing, with a conservative estimate of 12-15 million affected individuals in the United States of America (USA) by 2050 \(^7\). Importantly, it is associated with an increased mortality \(^8\text{-}^9\) and should therefore be considered a significant public health burden. Recent Australian data shows an exponential rise in AF
hospitalization, surpassing that of heart failure and approaching that of myocardial
infarction\textsuperscript{10}. In the USA, direct economic costs due to AF are estimated at $6 billion
annually\textsuperscript{11}. In the United Kingdom, AF accounts for 2.7\% of total national health
expenditure, 50\% of which is attributable to hospitalization alone\textsuperscript{12}. Although
population aging is regarded as an important contributor to this epidemic, obesity and
its associated co-morbidities may account for a substantial proportion of the increasing
prevalence of AF\textsuperscript{13}.

Epidemiological evidence has seen obesity emerge as a significant association with AF,
and this is particularly concerning in view of the projected rise in both epidemics\textsuperscript{14}.
Pathways through which obesity may promote AF remain obscured by coexisting
conditions; OSA, hypertension, diastolic dysfunction and other conditions promoting a
state of systemic pro-inflammation\textsuperscript{15-17}. Although left atrial (LA) structural remodeling
has been implicated as a common pathway promoting the AF substrate\textsuperscript{18}, components
of the proarrhythmic substrate itself remain poorly characterized.

**Obesity and atrial fibrillation**

The observation that body mass index (BMI) closely parallels the risk of AF has been
confirmed in several landmark epidemiological studies. Notably in 2007, Wanahita et al.
demonstrated in a meta-analysis, an associated 49\% increase in risk of AF in obese
compared to non-obese (overweight and normal BMI) individuals\textsuperscript{13}. In addition, the
authors showed a graded weight-dependent association; in the normal weight,
overweight and obese population, the relative risk of developing AF incrementally
increased from 1.3\% to 2.1\% to 3.2\% respectively. Utilizing data from the Framingham
Study into lifetime risk for AF\textsuperscript{18} and Olmstead County cohort projected risk, Miyasaka et al. suggested that 60% of the age and sex-adjusted rise in AF incidence may be accounted for by obesity alone\textsuperscript{7}. Using the Framingham cohort, Wang and colleagues provided a quantitative estimate; for every unit increase in BMI there was a corresponding 4% rise in risk of incident AF\textsuperscript{18}.

Beyond providing evidence via an epidemiological relationship between elevated BMI and the risk of new onset AF, in a further analysis of the Olmstead County longitudinal cohort projection data, Gersh et al. showed that progressive categorical weight gain, as defined by BMI, significantly influences the progression of AF from paroxysmal disease to more chronic forms of the disease\textsuperscript{19}. Furthermore, echocardiographic patient subset analysis suggested an augmented relationship between LA size and BMI, accounting for this progression. From this data, it is therefore plausible that excess adiposity (overweight and obesity) may modulate an electro-mechanical and electro-pathological atrial substrate promoting AF and allowing and its progression (paroxysmal to permanent). The pathways whereby obesity may promote AF remained unknown and obscured by multiple confounding variables.

A recent longitudinal prospective study by Tedrow et al. demonstrated a dynamic relationship between BMI and AF risk in women\textsuperscript{14}. Obesity was associated with short and long term increases in AF risk and this association remained significant despite adjusting for interim development of cardiovascular disease, diabetes and hypertension.
The study provided scope for the notion of weight management at potentially altering the course of the disease or curbing its epidemic progression.

**Obesity and left atrial size**

In the clinical setting, abnormalities in LA size are often observed in overweight and obese patients. Frequent uncertainty exists regarding the optimal method to standardize atrial size to body size, without eroding a potentially clinically important adverse relationship between adiposity and atrial dilation. Stritzke et al. conducted a 10 year follow-up study examining the relationship between weight change, hypertension, LA size and LA size indexed to height. The authors noted obesity as being a stronger independent predictor of LA enlargement, than the presence of hypertension. This relationship persisted following indexing of LA volume to height, and was compounded by the presence of hypertension. It is therefore noteworthy that to account for the LA volume changes with body size, but independent of obesity, the authors indexed to height rather than body surface area. Further, Garza et al. measured LA size (and height-indexed LA size) using echocardiography before and after (minimum 6 months follow-up) bariatric surgery-induced weight loss. Whilst minimal weight loss (mean 3kg) resulted in a further increase in LA size, profound weight loss (mean 43kg) resulted in a halted progression of LA dilation. This group difference persisted when LA was indexed to height. However the rigor of pharmacotherapeutic management (for example, the use of angiotensin active agents) or other coexisting conditions associated with atrial dilation (for example, sleep disordered breathing), remained unclear in this study.
Nevertheless, in view of the above observations from the Olmstead County data\textsuperscript{19} and the close relationship between obesity and LA size, LA dilation may represent the intermediate phenotype between obesity and AF.

Therefore, although numerous epidemiological studies have defined overweight and obesity as risk factors for AF, the histological and electrophysiological basis for this remains undefined. Therefore to define any atrial structural and functional changes paralleling weight gain, it is important to examine the current established models to study AF.

**Hypertension**

The relationship between hypertension and AF is perhaps the best studied. This stems from the observation that hypertension is the most prevalent condition associated with AF in community settings and therefore causation is plausible\textsuperscript{9}. Both the presence of hypertension and its severity are strong predictors of AF development in large prospective community cohort studies\textsuperscript{22, 23}. In addition, strict control of blood pressure (BP) appears to have a favorable disease modifying effect; specifically renin-angiotensin-aldosterone axis active agents may have a specific advantage secondary to their anti-fibrotic actions\textsuperscript{24, 25}. Although, such actions have been well established in the regression of adverse ventricular remodeling, it remains unclear whether additional similar mechanisms are in play at the level of the atrial myocardium. Previous small animal models of hypertension have employed a partial aortic clamping technique to study electro-structural changes in response to atrial stretch secondary to an elevated
afterload. In this study by Kim and investigators, the increase in afterload was shown to result in both hypertrophy and fibrosis of the LA with slow and inhomogeneous conduction velocity without changing the tissue effective refractory period (ERP) significantly. Accompanying the fibrotic and conduction change was a reduction in the expression of the putative gap junction protein Connexin-43. Moreover, earlier models of hypertension have described a similar substrate. Kistler et al. utilized maternal corticosteroid treatment to produce hypertensive off-spring. Atrial conduction mapping changes consisted of abnormal conduction wave front propagation and isochronal crowding. This resulted in electro-structural abnormalities principally consisted of slow inhomogeneous conduction, wave front crowding and finally, a greater AF inducibility accompanying a fibroblastic infiltration and fibrosis. Although illustrative of a hypertensive atrial substrate, a limitation relates to the extent at which such a model reflects the most common hypertension phenotype in humans – essential hypertension. Specifically, it is unknown if glucocorticoid-induced gene up regulation may confound the largely undefined phenotype of essential hypertension. To validate these observations, Lau et al. utilized a one-kidney one-clip model to investigate the impact of short and long term hypertension on atrial structure, function and proarrhythmia. Utilization of a one-kidney one-clip model intrinsically is more reflective of a disordered rennin-angiotensin axis and renal sodium handling – plausibly more in keeping with human hypertension. Long term (15 weeks) hypertension was shown to beget a higher burden (duration and episode) of AF, atrial fibrosis and inflammatory cellular infiltrates, conduction slowing, inhomogeneous conduction and elevated ERP.
These abnormalities were accompanied by LA dilation and reduced estimated LA conduit function (LA “ejection fraction”). Critically however, it was also demonstrated by the same investigators that short term (7 weeks) hypertension begets the substrate, thus highlighting the particular sensitivity of the atrial tissue to undergo adverse electro-structural remodeling, and in addition the potential importance of early hypertension identification and management. More recently, Medi et al. have conducted a mapping-based study of human hypertension, extending these observations of global conduction slowing and AF inducibility. However, the posterior right atrium and crista terminalis region appeared particularly vulnerable to hypertensive insult, demonstrating significant conduction delay. Although study of the atrial micro-structural substrate was not performed, the findings were congruent with those previously observed with the addition however of the role of the crista terminalis as an area of anatomic fiber bundle orientation non-uniformity, and therefore potentially vulnerable to anisotropic conduction. Similarly, Medi et al. studied the effects of primary pulmonary hypertension on right atrial remodeling, thus providing insights into the arrhythmogenic tendency of this condition – namely atrial flutter and to a lesser extent AF. Subjects with primary pulmonary hypertension had prolonged corrected sinus node recovery times (suggestive of sinus node dysfunction), lower atrial tissue voltages, greater atrial scar and slowed conduction and complex fractionated electrograms, although atrial tissue refractoriness was preserved. These findings suggest a common thread of atrial remodeling in various conditions predisposing to AF.
Therefore, although the perfect model to study atrial substrates subsequent to hypertensive damage is yet to be developed, the weight of evidence from animal models and human data currently supports an atrial cardiomyopathy consisting of hypertrophy and dilation, patchy fibrosis, conduction slowing, inhomogeneous conduction and wave front propagation thus predisposing to fibrillatory conduction.

**Obstructive sleep apnea**

Occult obstructive sleep apnea (OSA) may be an intermediary factor between AF and hypertension or obesity\(^\text{32}\). Furthermore, recent studies have shown a strong association between AF and OSA, independent of age, gender, hypertension, heart failure and BMI\(^\text{33}\). In the general community, Gami et al. utilized the Olmstead County cohort of more than 3000 patients referred for polysomnography, analyzing retrospectively for incident AF over a mean 4.7 years. Not only were the results concordant with the previous observation, but in addition there was also a strong association between OSA indices of sleep disordered breathing severity (apnea-hypopnea index, nocturnal arousal index, degree of oxygen de-saturation and nocturnal hypoxemia) and incident AF. Interestingly, this was noted only in the <65 year old age group. The authors concluded that mechanisms linking sleep disordered breathing and AF may be in addition to or independent of systemic arterial hypertension, hypoxemia-induced diastolic dysfunction, enhanced baseline sympathetic tone with enhanced automaticity, post-apnea parasympathetic augmentation with subsequent conduction slowing, coexistent pro-inflamatory state (evident by measurable serum markers such as high sensitivity

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C-reactive protein) causing atrial myocardial fibrosis and consequently areas of conduction wave front disturbances. Moreover, in a prospective study of 151 patients referred for direct current electrical cardioversion of AF and 312 free of past or present AF referred to a general cardiology clinic, the presence of OSA by the validated Berlin questionnaire was associated with an AF odds ratio 2.19, adjusted for BMI, neck circumference, hypertension and diabetes mellitus. Elevated BMI and OSA were powerful predictors of incident AF, independent of each other and this association between OSA and AF was more potent than conventional risk factors, such as hypertension alone. A further mechanism with dual consequences is intra-thoracic pressure changes which directly affect the cardiac chamber size through the process of stretch and autonomic tone changes. There is a subsequent measurable change in chamber size and possible stretch-activated ion channel conformational change – a mechanism proposed to represent mechano-electrical feedback. This may have implications at critical anatomic areas from which arrhythmogenesis may originate – namely the pulmonary veins. This interaction between intra thoracic pressure extremes and myocardial tissue conduction was investigated by Linz et al. Utilizing a pig model of OSA, the impact of negative thoracic pressure on early and late potassium channel conductance was studied, using AVE0118 (early), sotalol (late) and amiodarone (poly-channel blocker). Simulation of OSA resulted in shortening of the tissue refractory period and the increase AF inducibility. This effect was attenuated only after combined early (AVE0118) and late (sotalol) potassium channel blockade. It is plausible that atrial stretch through the process of extreme intra-thoracic pressure fluctuations may itself
promote conduction heterogeneity. To investigate this concept, Iwasaki et al. determined AF inducibility in a model of acute OSA in obese rats\textsuperscript{37}. Although difficult to extrapolate the findings to the chronic OSA clinical scenario, the investigators showed greater inducibility in obese rats with hypertrophic ventricles when OSA was induced. This effect was attenuated by pharmacological muscular paralysis and physical LA unloading. The findings suggest that perhaps OSA, through the process of acute atrial dilation/stretch, predisposes to AF in already remodeled hearts secondary to obesity. That is, obesity and OSA may be considered pre-requisites for AF, in this model. These structural changes may be reflected by the demonstrated functional abnormalities of conduction slowing, electrogram complexity and low atrial voltages. Despite the presence of reproducible epidemiological associations and observational studies, the mechanism linking sleep disordered breathing and AF remains obscure. Three interrelated mechanisms are worth exploring in detail; inflammation, hypertension and autonomic dysfunction. The presence of cyclic hypoxemia creates a pro-inflammatory state\textsuperscript{38} leading to endothelial dysfunction\textsuperscript{39}, specifically an imbalance between vasodilatory nitric oxide (NO) production and vasoconstricting endothelin-1 peptide (ET-1) production\textsuperscript{40}. In addition, cyclic hypoxemia promotes sympatho-vagal imbalance\textsuperscript{41}, which is thought to manifest as abnormal heart rate variability. There is a positive correlation between sleep disordered breathing, hypertension and systemic ET-1 peptide levels\textsuperscript{42} and in addition, the elevated ET-1 levels are ameliorated by Continuous Positive Airway Pressure (CPAP)\textsuperscript{43}. In a spontaneous hypertension-prone rat model, intermittent hypoxia was shown to enhance hypertension development through up
regulation of myocardial hypoxia-inducible factor-1 (HIF-1) – a promoter for the ET-1 gene. Furthermore, myocardial Endothelin (ET) peptide and its receptor were both up regulated, indicating a pathological ET system activation state. These changes were ameliorated using an ET receptor antagonist.

*Dietary sodium, pharmacotherapy and sleep apnea syndrome: Implications for hypertension*

Dietary sodium intake and excess weight are considered key risk factors for primary systemic arterial hypertension. In a study by Whelton et al., lifestyle measures to reduce sodium intake and increase physical exercise were compared, together and in isolation, to “general lifestyle measures” of brief advice\(^{44, 45}\). Sodium reduction to \(\leq 1.8\text{g/24 hours}\), as confirmed by urinary sodium, resulted in a mean systolic/diastolic BP (SBP/DBP) reduction of \((3.4\pm 0.8)/(1.9\pm 0.5)\) mmHg. Weight reduction of \(\geq 4.5\text{kg}\) resulted in mean decrease of \((4.0\pm 1.3)/(1.1\pm 0.8)\) mmHg and a combination of both was associated with a corresponding reduction of \((5.3\pm 1.2)/(3.4\pm 0.8)\) mmHg. This pattern of reduction confirmed the additive nature of lifestyle measures for effective BP lowering. Criticisms of the study however focused on the reliability of using a 24 hour urinary sodium ‘snapshot’ to determine the impact of dietary sodium intervention. The magnitude of BP lowering secondary to antihypertensive agents appears dependent on the pre-treatment BP level and the specific agent used.

Other studies specifically focused on dietary intervention for hypertension have demonstrated an approximate reduction of \(5.5/3.0\text{mmHg}\) using the DASH diet (high
fiber, vegetables and fruit and low fat dairy). Recently human and experimental studies have described an inverse relationship between dietary potassium and calcium intake, and more recently vitamin D, and BP levels. Although the relationship between weight loss and BP reduction appears linear, it is nevertheless modest. This modest effect may be secondary to the lack of efficacy of long term weight maintenance following weight reduction. In 2009, Kuller pointed out that it is important to know the trajectory of a subject’s weight in a weight-BP study prior to deriving a dose-response association. Specifically, a subject may lose substantial weight initially followed by slower weight gain and the periodic BP data collection may be unmatched to the true metabolic state at that time. Therefore, the current role of weight and lifestyle management in hypertension management appears to be supplementary to pharmacotherapy, but remains an integral component.

Pharmacotherapy therefore is central for the effective management of hypertension. Importantly, the responsiveness to a particular agent may depend on genetic factors (eg: African American individuals and their responses to diuretics verses calcium antagonists) or more importantly, co morbid conditions (angiotensin blockers in proteinuric renal disease, diabetes mellitus or left ventricular systolic dysfunction). The role of angiotensin in cardiac remodeling has focused interest on the use of renin-angiotensin-aldosterone axis active agents in AF. Beyond the antihypertensive effects, blockade of the pro fibrotic mediator may repress the atrial remodeling, and plausibly the conduction abnormalities. Studies into the efficacy of these agents in preventing AF
have indicated some benefit. In 2009, the GISSI-AF investigators studied the potential for valasartan in preventing AF and showed no benefit over placebo. Earlier however, Maggioni et al. demonstrated the benefit of Valasartan in reducing the occurrence of AF in patients with heart failure. Similarly, Healey et al. conducted a meta-analysis showing the comparable benefit of angiotensin II receptor blockers and angiotensin converting enzyme inhibitors in preventing AF in patients with LV systolic dysfunction.

Obstructive sleep apnea often coexists with obesity, hypertension and the metabolic syndrome. Furthermore, refractory hypertension can often be driven by underlying OSA. Therefore, much interest has focused on the potential for CPAP. In a recent placebo controlled cross-over study by Sharma et al., 3 months of CPAP therapy for OSA resulted in a mean 3.9/2.5 mmHg SBP/DBP lowering, improvement in lipid sub-fractions and levels of glycated hemoglobin. Overall, 13% of subjects developed reversal of the complete metabolic syndrome. The study had small numbers and key characteristics were not matched at baseline for the 2 populations, however if replicated the study results could have a significant impact on the metabolic syndrome in view of the high prevalence of both conditions.

**Cardiac failure**

A landmark study by Nattel et al. utilized ventricular tachy-pacing induced cardiac failure in a canine model to demonstrate atrial heterogeneity of tissue refractoriness in conjunction with patchy fibrosis. Extending these findings in the clinical setting by Sanders et al, atrial conduction was studied in a cohort of patients with cardiac failure.
and compared to a control group of patients. Conventional mapping demonstrated regional areas of conduction abnormalities with decreased conduction velocity, increased tissue refractoriness and prolonged sinus node recovery times. Electro-anatomic endocardial mapping demonstrated multiple pathological entities. This included areas of low voltage and electrical silence (signifying unhealthy myocardial tissue and electrical scar regional conduction prolongation), and fractionation/double-potential signals regionally, including the crista terminalis. These findings lend support to the intricate relationship between the structural substrate and the accompanying electrical changes. In addition to showing the same observed changes in animal models, this also provided translational evidence for the clinically observed sinus node dysfunction and AF often seen in various heart failure syndromes. Studies to date examining the atrial substrates in cardiac failure, promoting AF have utilized an induced structural defect (primarily mitral regurgitation and ventricular tachy-pacing cardiomyopathy). More recently, the novel use of anthracycline induced cardio-toxicity in a sheep model has allowed the study of atrial structural remodeling promoting AF, in the context of ventricular cardiomyopathy. The use of multi-site epicardial mapping and histology analysis showed a concurrence of atrial patchy collagen deposition and conduction abnormalities – remodeling of the “same sort”.

**Coronary disease**

Atrial fibrillation often complicates acute coronary events, increasing the risk of ischemic stroke and mortality. Preclinical studies in a canine model have shown
selective atrial ischemia to promote conduction slowing and delayed (hours) prolongation of tissue refractoriness, leading to a greater tendency to AF\textsuperscript{65}. The observation was made early by noting AF invariably occurred in the event of acute left circumflex artery obstruction proximal to the LA circumflex branch – thus suggesting atrial ischemia as the mechanism in play\textsuperscript{66}. This was further confirmed in a larger cohort of patients where LA branch occlusion of either the left or right coronary artery, were predictive of AF\textsuperscript{67}.

**Diabetes mellitus effect, metabolic syndrome and micro-vasculopathy**

Evidence of a specific pro-arrhythmic effect of diabetes mellitus (DM) has been conflicting; however recent large scale data has provided evidence to support a diabetes-specific effect in AF genesis. Retrospective case-control data by Movahed et al., utilizing more than 800,000 subjects from the Veterans Health Administration Hospitals showed using multi-variate analysis, that the presence of DM conferred a risk of AF or atrial flutter in the order of 2.13 and 2.20 respectively (expressed as odds ratios)\textsuperscript{68}. This was comparable to the 2.39 risk conferred by the presence of coronary heart disease – for which DM is a well-established potent risk factor. The authors hypothesized a role for “metabolic stress on the atrium” or microvasculature dysfunction with subsequent atrial ischemia. While the former may be explained in-part by the pro-inflammatory state accompanying DM, the latter was tested in an elegant study utilizing intracoronary doppler flow measurements, by Skalidis et al.\textsuperscript{69}. In that study, the authors compared the coronary flow characteristics in patients with lone
recurrent AF and those undergoing coronary angiography for other clinical reasons (for which no stenotic lesion was subsequently found) using specific coronary doppler flow velocity in both the left circumflex artery (LCx) and the LA branch of the LCx. This was performed at baseline and at adenosine-induced coronary hyperemia, to calculate coronary flow reserve. The authors demonstrated impairment of coronary flow reserve in the distribution of the LA branch in the LCx (not the LCx itself) in those with lone AF, suggestive of a potential micro vascular vasomotor dysfunction. It was proposed that a specific mechanism may exist for forming the substrate in “lone” AF, or at least a subset of that group. Although the study did not account for the component of DM itself, it is well established that DM is implicated in micro vascular coronary disease, the basis of which is likely endothelial dysfunction mediated. In another study utilizing coronary doppler wire and acetylcholine coronary reactivity, by Nitenberg et al., it was shown that patients with either type I or II DM (with angiographically normal epicardial vessels) have reduced coronary flow reserve and impaired vasoreactive responses (paradoxical constriction) to acetylcholine. Given the above evidence, DM appears to influence, if not create, the substrate by which AF occurs or perpetuates itself, again providing a mechanistic link between obesity or the metabolic syndrome and AF.

Clinically, the metabolic syndrome is a congregation of the following components: hypertension, abdominal obesity, low HDL, hypertriglyceridemia and impaired fasting glycemia. Chamberlain et al. utilized 15 year cohort study data from the Atherosclerosis Risk in communities Study to determine the relationship between AF and the metabolic
syndrome\textsuperscript{71}. The authors demonstrated a ‘dose-dependent’ incrementally cumulative risk for each added component, with the presence of all 5 conferring a 4.40 (95\% CI 3.25-5.94) relative to complete absence of the metabolic syndrome. Interestingly, the presence of hypertension was the most adverse component whereas hypertriglyceridemia did not confer any significant risk. To further characterize these atrial abnormalities Chang et al. utilized the electro-anatomic 3D mapping system (St. Jude Medical) to compare the LA substrate in patients with and without the metabolic syndrome\textsuperscript{72}. The findings of this study were that metabolic syndrome patients had larger LA sizes, higher incidence of non-pulmonary vein sources of AF triggers, shorter fractionated intervals, higher dominant frequencies, and complex fractionated atrial electrograms at the LA appendage base, coronary sinus and crista terminalis. In addition, patients with the metabolic syndrome had a higher failure rate following ablation of AF with increased arrhythmia recurrence.

The above findings add weight to the observation that structural abnormalities are integral to the conduction changes and the remodeling that perpetuated the arrhythmia.

Taken together, these collective findings suggest that intricately related to the morbidity of AF and failure of its treatment, are the risk factors predisposing to it in the first place. Management of such risk factors is likely to be central in the overall care of patients with AF.

\textit{Alcoholic atrial cardiomyopathy}
Various studies have investigated the effects of alcohol consumption on the cardiac conduction system. Although the effects of acute intoxication have been described through observational clinical studies and speculated to reflect an adrenergic surge, mechanistic studies have been sparse. Some investigators have proposed conspicuous alcohol consumption as a consequence of otherwise “lone” AF. Moreover, the impact of low dose yet chronic alcohol consumption on the thin delicate atrial walls remains largely unstudied. Kodama conducted a meta-analysis of 14 observational studies. Pooled estimates from a linear regression model showed a 1.08 increase in relative risk for every 10g alcohol consumed per day, along a risk continuum without a threshold effect.

A study by Cameli et al. studied the ECGs of 40 healthy subjects following the consumption of 5mg/kg red wine and fruit juice in separate experiments, with each subject also acting as their own control. The investigators found a prolongation of the surface P wave duration, QT duration and corrected QT interval. In addition, the AV node conduction time prolongation and amplification of its decremental properties were demonstrated in a canine model by Goodkind et al. Marcus et al. studied the relationship between atrial flutter and alcohol consumption. The authors showed a positive correlation between the odds of developing atrial tachyarrhythmia and level of alcohol consumption, in addition to progressive shortening of the right atrial effective refractory period. In contrast, Maki et al. studied the acute autonomic effects of alcohol consumption. The authors demonstrated changes in heart rate variability suggestive of a
heightened sympathetic to parasympathetic tone and a reduction in beta adrenoceptor
density, both suggestive of a heightened sympathetic response. Lai et al. examined the
ventricular remodeling in a murine model of chronic alcohol consumption. In addition
to ventricular myocardial cell death, the authors described redistribution and
quantitative changes in the connexin-43 gap junction protein. Electrophysiologic
changes included slowing of conduction and a greater burden of ventricular tachycardia
and ventricular fibrillation. Again with the paucity of data concerning atrial myocardial
consequences of chronic alcohol ingestion, it is tempting to extrapolate the mechanisms
of gap junction remodeling, conduction slowing and cell death underlying some atrial
arrhythmias. Conversely, it is conceivable or plausible that acute alcohol ingestion is
particularly toxic to the atrium, whereas chronic intake is particularly toxic to the
ventricles, and the atrial arrhythmias are secondary to elevated filling pressures. This
however remains speculative.

**Atrial remodeling in conditions predisposing to atrial myocardial stretch**

*Electro-mechanical remodeling in models of stretch*

Various clinical and preclinical models have been used to describe the remodeling
response of the pressure-overloaded atrium. These models have included the transverse
aortic constriction (TAC) rodent model, mitral valve stenosis (MS), mitral valve
regurgitation (MR), atrial septal defect (ASD) and left ventricular failure (LVF) with
elevated end-diastolic filling pressure. Certain common features have been described in
each of these experimental models. Roberts-Thomson et al. studied conduction changes
and voltage abnormalities\textsuperscript{79}. Subjects with ASD underwent electrical mapping in sinus rhythm and were compared to controls. Patients with ASD, showed increased LA volumes, prolonged P wave duration, and variable ERP – either unchanged or prolonged. There were also increased atrial conduction times, with regional slowing and frequent double potentials and complex fractionated electrograms. Fractionated complex electrograms are the electrical footprints of localized conduction block - an entity promoting circuitous wave front propagation. These changes translated to increased AF inducibility with programmed single extra stimulation. Mitral stenosis and its impact on LA and RA remodeling was studied by John et al\textsuperscript{80, 81}. This group undertook an elegant study utilizing MS patients scheduled for percutaneous mitral commissurotomy. They performed conduction studies and 3D voltage maps, in addition to AF induction by programmed electrical stimulation. Not surprisingly, MS patients had larger LA sizes, prolonged P wave duration and greater AF inducibility. Electrophysiological changes consisted of prolonged bi-atrial ERPs, reduced bi-atrial conduction velocities and conduction delay across the crista terminalis. With successful mitral commissurotomy, there was a reduction in atrial volumes, LA and pulmonary arterial pressures and P wave duration. Interestingly, there was a concomitant increase in bi-atrial conduction velocities and atrial voltages but no change in the ERP in the short term. Longer term, the electrophysiological changes progressively improved; P wave duration shortening, increased conduction velocities and atrial voltages and shortening of the ERPs. These changes suggest that, in this case, the arrhythmogenic substrate is perhaps amenable to ‘reverse remodeling’. Although no quantitative tissue analysis was
performed to analyze collagen content, the electrical abnormalities did show favorable changes with reversal/resolution of atrial stretch. Notwithstanding, these electrical footprints may therefore represent dynamic functional abnormalities rather than only structural. Roberts-Thomson et al. investigated intra operative epicardial conduction changes in 4 sets of patients\cite{82}; (i) Coronary artery bypass grafting surgery (CABG) with normal left ventricular (LV) function and normal LA size, (ii) severe aortic stenosis and normal LA size, (iii) CABG with severe LV dysfunction and enlarged LA size and (iv) severe MR with LA enlargement. The investigators used high density posterior LA epicardial mapping techniques to quantify conduction velocity, conduction heterogeneity, and conduction anisotropy and total plaque activation time. The data showed a functional line of conduction delay and block along a vertical line between the upper and lower pulmonary veins, with complex electrograms exclusively along that line. Pacing perpendicular to the line resulted in greater conduction slowing. This effect forces wave front detour and turn around, to bypass that line – thus setting the stage for re-entrant circuitous conduction and potentially fibrillatory conduction. These changes were amplified by the presence of atrial enlargement (groups (iii) and (iv)). The authors drew a link between the dynamic nature of electrical abnormalities; functional lines of conduction disturbances and underlying structural abnormalities. One hypothesis proposed is that the atrial posterior wall musculature layers are oriented in such a manner that normal areas of ‘crisscross’, forming heterogeneous wall thickness, may assume pathological conduction by amplifying anisotropy or possibly act as wavelet or ‘rotor’ anchors. To that end, Yamazaki et al. comprehensively investigated the...
complex relationship between atrial scroll waves to the regional variation in atrial wall thickness using a sheep Langendorff perfused hearts which have been either tachy-paced or not\textsuperscript{83}. Ex-vivo stretch induction was used to analyze the differences in scroll wave size and life cycle in the atria of tachy-paced and non-tachy paced atria. High resolution atrial epicardial and endocardial optical mapping was used to characterize atrial scroll waves and dominant frequency. They determined that atrial scroll waves are longer lasting, more numerous, more widespread in the tachy-paced hearts. In addition, these tend to anchor at anatomical sites of extreme atrial thickness transition. This work provides insight into the method by which electrical abnormalities, in the form of circuitous conduction, may interact with underlying ‘natural’ anatomical structural substrates.

\textit{Molecular mechanisms underlying the remodeling process in atrial stretch}

The above concepts of altered conduction times, tissue refractoriness and functional lines of block have been proposed to occur due to the heterogeneity of the effect of stretch on the different atrial regions\textsuperscript{84}. Essentially, stretch is a non-uniform process probably by the very nature of the complex atrial chamber anatomy. Why the LA posterior wall is most vulnerable to stretch remains unclear. One plausible explanation stems from the observation that structurally, the posterior wall is the thinnest section of the LA. Furthermore, the posterior wall and the pulmonary veins originate embryologically from the same progenitor cells. The pulmonary veins (structures constantly undergoing phasic stretch) have an established role in the pathogenesis of AF
and as a result, there may be an intrinsic myocardial predisposition to electrical remodeling under conditions of stretch. Ionic channels suggested to play a role in the electrical conduction and refractoriness heterogeneity are particularly the sodium-calcium exchanger channels, which may in addition contribute to ectopic impulse formation and therefore electrical triggers. In addition, there is an apparent increase in a pro-fibrotic milieu.

Adiposity and the heart

‘Lipotoxic cardiomyopathy’

Aside from considering systemic effects of obesity and the metabolic syndrome and its relationship to AF, the effect of local fat stores contiguous to pericardial adipose tissue as an endocrine and/or paracrine organ system has been highlighted. Although systemic levels of triglycerides have been known to influence myocardial structure and function, the relationship to cardiac pathology been only recently been recognized. Lipotoxic heart disease has been used to describe the ventricular myocardial apoptotic changes shown in a leptin receptor deficient rodent model of obesity, inducing myocardial tissue lipidosis.

Pericardial fat

Perhaps similar to the observations in the ventricles, support for a local mechanism occurring in the atria was demonstrated through several observational studies. Akar et al. showed incident AF to be positively correlated with pericardial fat volume as
quantified on computed tomography (CT)\textsuperscript{91}. In addition, there appeared to be a graded relationship with a higher pericardial fat burden in persistent AF patients compared to paroxysmal AF patients. This relationship was independent of traditional measures of obesity, specifically BMI. Thanassoulis et al. utilized the Framingham cohort to show the independent predictive power of pericardial fat, but not visceral or intra thoracic fat depots, for the prevalence of AF\textsuperscript{92}. In addition, Wong et al. demonstrated the impact of pericardial fat on chronicity and severity of AF\textsuperscript{93}. Poor outcomes following catheter ablation of AF were also associated with higher burdens of pericardial fat, and this relationship persisted despite statistical adjustment for traditional measures for BMI. Currently there remains a mechanistic gap demonstrating the suspected paracrine toxic effect to explain these observed associations. However, in separate investigations, Batal and Wong\textsuperscript{94,93} quantified local peri-atrial fat and its relationship to AF. Although a local paracrine mechanism remains persuasive, this remains unproven. Furthermore, standardized pericardial fat measurement techniques, ideal imaging and external validity remain undetermined. Finally, the dynamics of pericardial fat changes with whole body size and adiposity changes, and this relationship to AF, requires further research.

*Adiposity and Inflammation*

Inflammation has been proposed as the intermediary between contiguous or systemic adiposity and atrial remodeling, predisposing to AF. Although pertinent to coronary atherosclerosis, Hirata et al. demonstrated an activated macrophage phenotype in
pericardial fatty tissue, when compared to subcutaneous fat. The presence of tissue inflammation in various models of atrial stretch depends on the underlying disease state and its chronicity (short term hypertension has been associated with atrial inflammation). The importance of inflammation and particularly neutrophil myeloperoxidase was recently shown in a mice gene knock out investigation. The study demonstrated the role of neutrophil myeloperoxidase in micro-architectural atrial tissue damage and subsequent conduction changes. Clinical translation of this concept was shown by successfully employing colchicine (neutrophil chemotaxis inhibitor) for post-operative AF prevention. A plausible mechanism of post-operative AF is therefore peri-operative fluid shifts and hemodynamic changes contributing to atrial stretch and subsequently inflammation-induced structural remodeling and consequentially conduction changes.

**Myocardial energetics and fuel utilization**

Investigators have studied the sensitivity of myocardial tissue fuel utilization mechanisms, structure and function to various dietary modulations. Adult myocardium primarily utilizes fatty acid oxidation for energy production in preference to glucose. This situation is opposite in the fetal heart, and normal myocardium adapts to a high fat diet by increasing fatty acid β-oxidation correspondingly and subsequently accumulating ketone bodies. Yan et al. used a rat model to genetically manipulate fuel transportation, by over-expressing the insulin-independent cardiac specific GLUT1 glucose uptake transporter. These genetically manipulated rats utilized glucose as fuel...
and continued to do so, despite a high fat obesogenic diet. This glucose-toxicity leads to ‘metabolic remodeling’ with further down regulation of, and a shift away from, fatty acid oxidation. That is, glycolytic pathways are up regulated to ‘cope’ with the intracellular hyperglycemia. Acyl-CoA enzymes responsible for chaperoning fatty acids across the mitochondrial membrane, are down regulated and fatty acids accumulate in vesicles in the cytosol. This was found to contribute to contractile dysfunction.

Translational work by Gropler et al. showed increased uptake and oxidation of $^{11}$C labeled palmitic acid in obese females with DM$^{100}$. Excess lipid availability of the heart limits its mitochondrial utilization thus diverting metabolites to esterified derivatives (di and tri acetyl glycerol and ceramides) accumulating in the cytosol.

Accordingly, mounting evidence has strengthened the concept that dissociated myocardial fatty acid uptake and oxidation lead to structural and functional changes. In addition to the observation that induced cellular ‘gluco-toxicity’ or hyperglycemic stress leads to impaired fatty acid oxidation and cellular lipid accumulation, it is recognized that myocardial β-oxidation inhibits glucose uptake and utilization thus contributing to further glucose intolerance$^{101}$. Such insights demonstrate the interplay between diet, myocardial fuel utilization and myocardial function translating to contractile abnormalities. Indeed, intra-myocardial lipidosis is observed in obese and insulin resistant patients. However, this observation is often confounded by the underlying cause of the subject death, as these are obtained from post-mortem examinations and is therefore limited to magnetic resonance spectroscopy imaging studies$^{102}$. 

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Overview of atrial structural remodeling

Various models have been used to study the process of structural remodeling in AF. These have primarily included animal models of rapid right atrial pacing (RAP), congestive heart failure (CCF), MR and methacholine-induced AF. The RAP model utilizes constant rate rapid right atrial pacing with a normal constant rate ventricular rate (not paced). The CCF model is induced through rapid ventricular pacing to induce ‘tachycardia-mediated cardiomyopathy’ with subsequently elevated filling and atrial pressures. The MR model is subsequent to mitral chordae avulsion resulting in atrial stretch secondary to significant regurgitation. The methacholine model attempts to replicate parasympathetically or vagal induced AF, through intra pericardial methacholine injection. More recently, knockout mouse models have attempted to isolate the significance of a structural or functional element. Furthermore, the apparent conduction abnormalities observed appear to relate to the underlying pathological model studied. Verheule et al. studied the electrical differences between the RAP and MR models of AF in canines. The MR model demonstrated greater regional conduction slowing and increased conduction heterogeneity, which was dependent on the direction of pacing with preferential pathway conduction and evidence of conduction block. Furthermore, there was a functional component to these conduction changes seen in the MR model. With faster pacing or addition of prematurity there was an amplification of these abnormalities. Investigations into the structural substrate have revealed certain features which have been described as “de-
differentiation” or a resumption of a myocellular fetal phenotype. Alternatively, these changes may represent a hibernating myocardial phenotype. Either way, these changes have been proposed to represent an attempt at more efficient cellular fuel utilization. These micro-architectural changes have included contractile elements, cellular structural elements and cellular fuel utilization and nuclear elements. This specifically included sarcomeric loss, glycogenosis, reduced cytoskeletal cardiotin and increased alpha smooth muscle actin. In addition, changes in mitochondrial membrane folding have been observed. In these goat models, the structural “de-differentiation” occurred over a period of several months and their reversal was slower and incomplete. Human models of AF have been more limited. Atrial septal biopsies from patients with lone AF have demonstrated changes more in keeping with degenerative pathology with inflammatory cellular infiltrates, myolysis and replacement fibrosis. Furthermore, these structural changes may be spatially heterogeneous and non-uniform in severity and distribution, contributing to the atrial contractile abnormalities often observed in AF.

**Overview of atrial mechanical functional remodeling**

The slower regression of the profound structural abnormalities discussed in the previous section may have direct functional consequences for the atria following the establishment of sinus rhythm. Although non-uniformly observed, mechanical dysfunction in the form of diminished atrial echocardiographic A wave, appears to positively correlate with the preceding length of sustained arrhythmia. It is
plausible that this slow recovery of ‘atrial systole’ is secondary to the hibernating phenotype and sarcomere loss. Additionally, these changes may account for the persistent pro-thrombotic state post-cardioversion\textsuperscript{115}. In a goat tachycardia mediated cardiomyopathy model, Scotten et al. showed that the work index (atrial pressure-atrial diameter loops) declined following 12 hours of atrial tachy-pacing and a complete diminishing of the work index following 48 hours\textsuperscript{116}, illustrating a tachycardia duration-dependent effect on atrial mechanical function. Furthermore, the investigators demonstrated the underlying electrical remodeling process, specifically depressed calcium channel conductance, which predisposes to the mechanical abnormalities.

**Overview of atrial electrical remodeling**

The concept of atrial remodeling derives from the observation that RAP leads to abbreviation of the erective refractory period of that tissue and a predisposition to further spontaneous AF\textsuperscript{117,118}. The duration of AF was directly related the pre-existing duration of atrial tachy-pacing. In addition to ionic and gap junction remodeling (described below) resulting in conduction slowing, the atrial functional re-entry wavelength shortens. Nattel et al. proposes that this shortening of the re-entry wavelength (refractory period and conduction velocity product) permits the sustenance of multiple re-entry circuits within the confines of the anatomical atrial area\textsuperscript{119}. Furthermore these electrical changes lead to a loss of normal rate-adaptive changes in the refractory period\textsuperscript{120}. Correspondingly, loss of rate adaptation appears to cluster with a high AF burden, in human studies of AF\textsuperscript{121}.
Our current understanding of atrial electrical remodeling is that it is primarily the result of interplay between potassium channel function, calcium conductance changes and gap junction protein remodeling.

**Potassium currents**

Changes in potassium channel function may be best described as heterogeneous with differences between the different subtypes; voltage-dependent or rectifier types, as well as mechanisms of down regulation; reduced channel function or protein component expression\textsuperscript{120, 122, 123}. Furthermore, the impact of these acquired potassium channelopathies on action potential duration remains unclear, as they are thought to not be major contributors\textsuperscript{124}. Cholinergic AF, on the other hand may have its basis in the up regulation in acetylcholine sensitive rectifier potassium channels observed in canines\textsuperscript{125, 126}. Although recent functional studies have suggested an importance of potassium conductance in human AF\textsuperscript{127}, the conflicting overall results indicate a need for future studies to clarify their role in different underlying disease models.

Inward rectifier potassium channels generally function to shift the resting membrane potential away from the depolarization threshold, making it therefore, more negative\textsuperscript{128}. Some studies have demonstrated an increase in the function of these channels in the setting of AF\textsuperscript{128-130}.

**Calcium currents**
Calcium conductance changes have been better characterized in the AF electrical remodeling process. Depolarization and contractile induced increased L-channel calcium conductance, through rapid atrial rates, results in myocellular calcium overload. As a cellular protective mechanism, L-type channels appear to be functionally down regulated, followed by structural or quantitative decreases. However, this cellular defense against calcium overload is at the expense of further electrical anomalies that promote re-entry circuit sustenance, through cellular action potential and refractory period abbreviation\textsuperscript{120, 129}. These alterations in calcium channel kinetics have been delineated through the use of pharmacological probes – nifedipine and experimental calcium channel agonists, by the same investigators\textsuperscript{120}, thus illustrating proof-of-concept. The importance of the acute electrical changes have been noted to reverse relatively rapidly (compared to the abovementioned structural and myocellular changes) following the restoration of sinus rhythm\textsuperscript{131}. In addition, these altered calcium transit currents have three fold consequences; beyond promoting further electrical promotion for re-entry, there appears to be a role for contractile or mechanical dysfunction and possible diastolic triggered activity\textsuperscript{132, 133}.

\textit{Sodium currents}

Genetic features of familial AF has provided some insight into the potential importance of sodium currents\textsuperscript{134}. Conceptually, down regulation of channel protein sub-unit components or channel function may underlie conduction slowing\textsuperscript{135} and therefore promoting re-entry, however such findings have varied in accordance with the underling
model, time-course of AF (or tachy-pacing) and species studied\textsuperscript{136}. Comparatively, sodium channel function in AF is less well characterized\textsuperscript{137}.

\textit{Electrical gap junction changes}

Connexin gap junction proteins 40 and 43 and their influence on atrial vulnerability have been studied using various models, and certainly this may have a clinical basis\textsuperscript{138}. Overall the studies have not been conclusive with regards to whether an increase or decrease is observed in either protein. Nonetheless, the consistent finding appears to be the spatial heterogeneity and the relative changes in the two protein sub-types\textsuperscript{139, 140}. It is plausible that these changes may underlie the observation of increased conduction heterogeneity and anisotropic conduction.

\textit{Summary of ionic, structural, functional and metabolic remodeling in atrial fibrillation}

Therefore, with the recognition of our incomplete understanding of the various components of the ionic remodeling process, a proposed summary of the above concepts may be as follows; In AF, there are profound and heterogeneous potassium current alterations with decreased calcium conductance as a response to intra cellular calcium overload. There is also dysfunction of intra cellular calcium regulating mechanisms. In addition there are heterogeneous alterations in sodium conductance but more importantly ‘lateralization’ or ‘functional dissociation’ of connexin gap junction proteins. This leads to zigzag conduction, wavefront breakdown and areas of conduction block. These changes lead to regional slowing of conduction and
concurrently, prolongation of action potential duration and shortening of the refractory period. Such electrical changes promote re-entry through increasing atrial wavelength and subsequent stabilization of the ‘rotor(s)’. Electrical remodeling appears rapidly with the onset of AF and reverse within a comparable time frame. Calcium abnormalities lead to contractile and mechanical dysfunction (therefore effort intolerance and pro-thrombotic state) and enhanced triggered activity. In conjunction, there are profound metabolic changes regulating fatty acid and glucose utilization, leading to toxic intracellular byproduct build up. Cellular ultra-structural changes appear later than electrical changes, during the time course of the remodeling process. Micro-architectural changes include loss of the contractile apparatus and cell structural elements as well as mitochondrial and nuclear chromatic morphological abnormalities – intimately linked with the cellular metabolic changes. Over time, replacement and interstitial collagen deposition occurs, and myocardial damage ensues. The presence of ongoing triggers, coupled with fibrosis and abnormal connexin distribution, leads to the self-perpetuation and progression of AF – changes which have collectively been dubbed “the second factor”\textsuperscript{60}. It is these structural changes shown at least partial, but much slower, recovery.

\textit{Summary of the temporal events of the remodeling process in response to atrial fibrillation}

In the immediate term, atrial myocardial energetics and fuel utilization undergo adaptation changes in response to the increased metabolic demand of the atrium. There
is a switch to glycolysis. Coronary perfusion and oxygen extraction increase to meet contractile demands, resulting in increased intracellular calcium and sodium, and increased sarcoplasmic calcium content. Elevated intra cellular calcium leads to down regulation of calcium channel conductance, resulting in suppression of atrial contractile force and conduit function. The suppressed calcium conductance through L-type channels leads to cellular action potential and refractory period abbreviation. These effects may be mediated by either a decline in calcium channel function and/or a quantitative reduction in channel protein. The acute electrical remodeling process is thought to encompass several days, whereby a new state of tissue refractoriness is reached\textsuperscript{117}. The resulting continued fibrillation will therefore promote conduction velocity slowing. As a result, the combined shortening of refractoriness and conduction slowing results in the increased probability of fibrillatory conduction and a low probability of spontaneous rotor extinction. It appears that this phase is largely reversible over the same time course from which it occurred.

With further fibrillation however, additional mechanisms are involved. The hallmarks of this stage include myocardial structural, functional and metabolic changes; principally cellular micro architectural and tissue structural abnormalities. This period is thought to span a period of ‘months’ of arrhythmic adaptation, and in turn may take longer to reverse following the termination of AF. Furthermore, AF by this stage may be more resistant to cardioversion by either electrical or pharmacological means. Specifically, these changes are primarily de-differentiation, myolysis, connexin-40 spatial re-
distribution, sarcomeric loss, cell hypertrophy, mitochondrial morphological changes and cytosolic glycogen accumulation. Importantly, extra cellular matrix remodeling and peri-muscular tissue fibrosis appears to become organized. With the ongoing calcium conductance dysregulation, there is interference with the sodium-calcium membrane exchanger, resulting in a greater propensity towards triggered activity. This latter finding may be particularly crucial in certain disease states, such as cardiac failure, or act as sources of ongoing arrhythmic triggers that sustain the AF. Notwithstanding, these 2 stages must not be considered as separate entities. Indeed, there is vertical integration, with the latter structural remodeling (phenotypically, atrial dilation) interacting with the former processes promoting wavelet stability, henceforth resulting in greater begetting of AF. This is vaguely dubbed as the “second factor” and as previously highlighted, promotes the transition of paroxysmal AF to more chronic persistent forms and renders the arrhythmia resistant to electrical cardioversion in the long term, beyond that seen early in the disease due to acute electrical remodeling.

The pro-fibrotic milieu

Collagen deposition and fibrosis appears to be a consistent finding in studies defining the micro-architectural substrate for AF. Atrial fibrogenesis appears to be processes resulting from a variety of insults on the thin delicate atrial tissue, with multiple inter related biochemical pathways. It is likely that these electrophysiological atrial myocardial fibrotic changes may contribute to abnormal conduction and/or the assumption of trigger activity for arrhythmogenesis. In addition, there is evidence that
fibroblasts, the functional units of tissue fibrosis, can assume electrical activity and accordingly, contribute and stretch induced electro-mechanical feedback\textsuperscript{141-143}. Therefore, the process of fibrosis may be more than merely a structural barrier to conduction.

Most of our current understanding of extracellular matrix (ECM) deposition in pathological states has been through the study of ventricular disease and different mechanisms may be in-play depending on the underlying pathophysiologic state; infarction (ischemic cardiomyopathy), pressure-overload (valvular, hypertensive), infiltrative disease (fibro-fatty infiltration, granulomatous), etc. Under the premise that collagen deposition is matched by cardiomyocyte hypertrophy in non-pathological states, a diseased state results from an ECM to functional cardiomyocyte ratio mismatch under the influence of autocrine, paracrine and endocrine signals\textsuperscript{144}. The phenotypic transition of a fibroblast to an active myofibroblast is modulation by a variety of mediators such as, transforming growth factor beta (TGF-\(\beta\)), platelet derived growth factor, angiotensin II, aldosterone and Endothelin-1 (ET-1)\textsuperscript{87, 143, 145}. These signaling systems have been the focus of much investigation given the potential for pharmacological targeting or modulation of the fibrosis functional unit.

\textit{TGF-\(\beta\)}

Transforming growth factor beta (TGF-\(\beta\)) exists in 3 interrelated isoforms (TGF-\(\beta\)1, TGF-\(\beta\)2 and TGF-\(\beta\)3), all ligands for the serine/threonine kinase receptor and each exhibiting a subtly unique but yet fully undefined role\textsuperscript{143}. This cytokine is particularly central to the
fibrotic process particularly in pressure-overloaded hearts. Upon binding of TGF-β to its kinase receptor, a variety of intracellular signals are activated. Cytoplasmic Smad proteins have been characterized as mediating downstream intracellular signals. These proteins are activated and initiate nuclear transcription of collagen and fibrosis encoding genes. Most of the current understanding of its central role emanates from preclinical work utilizing small animal aortic constriction model and gene knockout studies or cell culture lines. In vitro human atrial cells exposed to tachy-pacing resulted in reactive oxygen species (ROS) generation and myofibril degradation. Conversely, molecular interference with TGF-β signaling ameliorated markers of ROS and myofibril degradation.

Angiotensin II

Angiotensin II is a peptide hormone derived from angiotensinogen, an α2-globulin, by way of renin and angiotensin converting enzyme sequential cleavage, in renal and pulmonary tissue. Angiotensin II has widely known pro-fibrotic and vasomotor effects as well as endocrine activity on the potent pro-fibrotic, sodium conserving and dipsogenic mineralocorticoid, aldosterone. Correlative human studies using pharmacological targeting of the angiotensin-aldosterone system suggests that it has an inductive effect on the expression TGF-β. It is observed that the particular benefit of various pharmacologic agents in halting adverse remodeling is related to their anti-fibrotic actions by interfering with TGF-β or its downstream signaling cascade. Furthermore,
angiotensin II and ET-1 independently modulate the hypertensive pro-fibrotic response\textsuperscript{149}. Specifically, the fibrogenic effects of angiotensin II are dependent on ET-1.

*Connective tissue growth factor*

Connective tissue growth factor (CTGF) is a heparin binding cysteine rich matrix associated protein targeting a wide variety of tissues, including atrial myocardium. It appears to illicit a variety of changes such as proliferation, fibrosis, apoptosis, cellular adhesion and angiogenesis\textsuperscript{150}. Connective tissue growth factor appears critical in the differentiation process and phenotypic commitment of mesenchymal cells to fibroblasts\textsuperscript{151}. In addition, CTGF has a particular role in affecting endothelial cell growth, adhesion and migration as well as interacting with vascular endothelial growth factor (VEGF)\textsuperscript{152}. In a sense, CTGF functions as a chemotactic factor with what is likely a large receptor profile, or a ‘localized chemokine’, given that a CTGF specific receptor has not been identified. In healthy states, CTGF is constitutively expressed on cardiac fibroblasts whereas in diseased states, CTGF is also expressed on cardiomyocytes. It is at least under dual control of TGF-\(\beta\) (post-transcriptional effector ligand) and ET-1 and it appears that CTGF actions closely mirror TGF-\(\beta\) and parallel those of ET-1\textsuperscript{153}. Perhaps the most compelling evidence of a role in atrial fibrosis came from gene silencing studies\textsuperscript{154}. Endothelin-1 up regulates CTGF expression in atrial myocardium and the *in-vitro* pro-fibrotic effects of ET-1 were abolished by CTGF gene silencing. In addition, CTGF expression has been shown to be under Angiotensin II control\textsuperscript{155, 156}. Taken
together, it is likely that CTGF is involved in physiological and pathophysiologic states of wound healing and fibrogenesis, respectively.

*Platelet derived growth factor*

Platelet derived growth factor (PDGF) is a growth factor protein critical in cell proliferation, growth and migration of mesenchymal and smooth muscle cells as well as angiogenesis. It is widely produced by a variety of cells, including endothelial cells. The protein is a dimeric of A-A, B-B or A-B subunits with tyrosine kinase receptors modulating downstream nuclear transcription of signaling pathways involved in fibroblast mitogenesis (G1 checkpoint skipping) and fibrogenesis\(^{157}\). The importance of PDGF in the particular susceptibility of atrial tissue to metabolic and hemodynamic insults, relative to ventricular myocardium, was illustrated by Dr Nattel’s group\(^ {158}\). Gene microarray studies have identified PDGF and its receptor as particularly abundant in atrial tissue relative to ventricular tissue and these differences were exaggerated by the presence of cardiac failure. In this same work, using plated atrial and ventricular fibroblasts, thymidine uptake (marker of proliferation) was compared between both cell origins, to a variety of mediators; Fibroblast growth factor-β, TGF-β, ET-1, angiotensin-II and PDGF-AB. Platelet derived growth factor was shown to illicit a more atrial-specific fibroblast activation. Further, the addition of a PDGF tyrosine kinase receptor inhibitor abolished this atrial-ventricular susceptibility differential. The authors concluded that PDGF could act as a novel and potentially atria-specific target by pharmacologic modulation. To investigate the role of the PDGF-mast cell axis, tissue infiltration of mast
cells and AF susceptibility in response to TAC was assessed before and following the introduction of cromoglycate (a mast cell stabilizer) then PDGF receptor (α subtype) neutralizing antibody. Mast cell infiltration and fibrosis in atrial tissue was prominent in TAC rats and ameliorated by cromoglycate. In addition, AF susceptibility and fibrosis was promoted by the addition of PDGF-A ligand and abolished by the introduction of the receptor neutralizing antibody, thus providing proof of the specific role of PDGF-A from mast cells and its interaction with PDGF receptor α-subtype, in AF susceptibility and atrial fibrosis, in a pressure overloaded in-vivo cardiac model\(^{159}\).

*Endothelin*

1. **Overview of Endothelin peptides and their receptors**

Endothelins are relatively small peptides with 3 main subtypes; ET-1, ET-2 and ET-3, each derived from its corresponding “big” parent, and metabolized by the endothelial cell derived Endothelin converting enzymes (ECE), forming active ligands. Endothelins are very potent vasoconstrictors balancing homeostatic vasomotor mechanisms with vasodilatory NO and prostacyclin. Its expression is particularly prominent in the vascular system, myocardium and vascular adventitial fibroblasts. Endothelin-1 is expressed on vascular endothelial cells, cardiomyocytes, fibroblasts and smooth muscle cells. The ligand interacts with three G-protein coupled receptor subtypes (ET-A, ET-B1 and ET-B2). Though unclear, the interrelationship between ET-A and ET-B appears reciprocal and auto-regulatory. In adventitial fibroblasts, ET-A receptors mediate the agonist function of ET-1, whereas ET-B mediates the clearance of the ligand, ET-1\(^{160}\).
The mitogenic and vasomotor effects of ET are induced by a variety of factors; inflammatory cytokines, hypoxemic conditions and oxidized LDL. Broadly, the endothelin system functions in vascular and ECM remodeling, beyond vasomotor control. The ET-ET receptor axis has been implicated in systemic hypertension\textsuperscript{161}, obesity and the metabolic syndrome\textsuperscript{162}, cardiac hypertrophy\textsuperscript{95, 163}, arterial disease\textsuperscript{164} and possibly sleep apnea (through hypoxic insult)\textsuperscript{42}. One early observation illustrating a central pathophysiologic role of ET-1 in fibrotic diseases is between scleroderma\textsuperscript{165} and diabetic cardiomyopathy\textsuperscript{166}. Markers validating the excess activity of ET-1 and its influence on fibrosis have led to clinical exploitation of this pathway in digital ulceration disease. By corollary, using a streptozocin diabetic mice model, ‘hyper-endothelinemia’ was associated with myocardial fibroblastic recruitment via endothelial to mesenchymal transition and cardiac fibrosis and this effect was abolished in the ET-1 knockout mice\textsuperscript{166}.

2. Endothelin axis, obesity and metabolic syndrome

Starting from the late 1990’s, investigators have increasingly recognized the ubiquitous nature of Endothelin signaling in obesity alone in addition to individual components of the metabolic syndrome, through various clinical and preclinical studies, providing persuasive evidence and utilizing proof-of-concept antagonist studies\textsuperscript{167-170}. Nevertheless, the picture remains far from complete in so far as the lack of a current therapeutic benefit emerging from the proposed mechanisms, attesting to our incomplete understanding of this pathway.
Levels of the ET-1 ligand are elevated in obese patients. Small animal studies have suggested a post-receptor interaction between Endothelin-1 and insulin metabolic and mitogenic effects\textsuperscript{171}. Eriksson at al. investigated the effect of ET-1 on insulin-induced anti-lipolytic effects in obese subjects\textsuperscript{172}. In-vitro incubation of human cultured subcutaneous adipocytes with ET-1 stimulated lipolysis in a dose-dependent fashion. This effect was replicated by the addition ET-A specific receptor agonist, but not an ET-B receptor agonist. Correspondingly, ET-A receptor protein levels were significantly increased in subcutaneous adipose tissue as quantified by western blotting, but not on mRNA quantitative analysis – implying post transcriptional modulation responsible for protein up regulation. Another potential operative mechanism of ET signaling in obesity may relate to vasomotor endothelial dysfunction. Mather et al. tested the hypothesis that elevated ET-1 in obese subjects (with and without DM) is responsible for the unfavorable interaction with endothelial derived relaxing factor mechanisms\textsuperscript{173}. Methacholine is an acetylcholine analogue used to study endothelial cells’ response in producing NO (Endothelial derived relaxing factor, EDRF) and the experimental ET-A receptor blocker, BQ123, is used to determine specific ET system signaling. Administration of BQ123 resulted in increased peripheral blood flow in the obese and DM groups without changing the basal production of NO flux, thus assuming a correction of the underlying basal vasoconstriction due to increased ET-1 ligand as opposed to increasing EDRF/NO levels. In addition to basal condition, methacholine stimulated NO production was augmented by the co-administration of BQ123 in obesity/DM, suggesting that Endothelin blockade corrects the obesity/diabetic induced
vasomotor dysfunction and permits the (protective) vasodilatory effects of EDRF/NO. Lteif et al. proposed a similar mechanism in play between ET-1 and the impaired insulin-induced vasodilation and glucose extraction in obese/insulin resistant patients\textsuperscript{174}. Again, this dysfunction was ameliorated by the administration of BQ123. These effects were shown in obese subjects, but not lean subjects.

The recognition of ET-1 abnormalities in obesity and its known vasoconstrictive function has focused research into its potentially mediating effect in the relationship between obesity and systemic arterial hypertension and ventricular mass\textsuperscript{175}. Furthermore, levels of ET-1 have been shown to predict the development of systemic arterial hypertension in a normotensive cohort over mean a follow-up period of 7 years\textsuperscript{176}. This relationship appeared to be additional to the effect of such confounder as; BMI, age, renal function, baseline systolic blood pressure and level of alcohol consumption. In addition, Belaidi et al. investigated the interrelationship between hypertension and cyclic hypoxemia of sleep disordered breathing\textsuperscript{42}. The group used a spontaneous hypertensive rat model and intermittent hypoxia (IH), effects on blood pressure and the relationship to ET-1. Chronic IH further promoted hypertension in this rat model, and was associated with elevated myocardial pro-ET-1, ET-1 and the ET-A receptor levels. The authors proposed Hypoxia-inducible factor-1, as the ET-1 transcription promoter. Finally, administration of Bosantan (mixed ET-A/B receptor antagonist) reduced the hypertension augmentation by IH. This was therefore proposed as a plausible mechanism in the sleep apnea-hypertension link.
3. Endothelin axis and cellular arrhythmogenesis

The cytokine signaling network and its role in promoting arrhythmogenic conduction and atrial remodeling is an area of active research. In an elegant study by Reisner et al. utilizing isolated rat ventricular cardiomyocytes, cellular conduction and structural changes were investigated in the absence of, and following incubation with ET-1 peptide\(^{177}\). Compared to control cells, ET-1 exposed cells exhibited structural remodeling with cellular hypertrophy, decreased Connexin-43 gap junction proteins and increased Connexin-43 total protein. Functionally, ET-1 treated cells showed diminished excitability to micro-electrode array pacing stimulation and slowing of wave front propagation on isochronal activation mapping. Although the data was pertinent to ventricular myocytes and therefore extrapolation to atrial tissue must be guarded, taken collectively in the context of previous research, this provides persuasive evidence for the role of ET-1 in cardiac electro-structural remodeling. Further mechanistic studies have strengthened the hypothesis. Using an isolated rat atrium model, Endothelin receptors and Endothelin converting enzymes were shown to be expressed at greater levels compared to ventricular myocardium\(^{178}\). Following the induction of in-vitro stretch, Mitogen Activated Protein Kinases (MAPKs) and their downstream mediators were increased along with a profound increase in ET-1 mRNA levels, and this correlated with the atrial hypertrophic response related genes. These effects were attenuated following Bosantan (ET-A/B receptor antagonist) administration. These concepts have been furthered in a transitional observational study looking at the quantitative
association between atrial rhythm, size and Endothelin-1/ET receptors\textsuperscript{179}. Using intraoperative atrial appendage sampled the investigators demonstrated a positive correlation between ET-1 and atrial size, persistence of AF, severity of MR and presence of cardiac failure. Multivariate analysis showed ET-1 levels associated with presence of AF, atrial size and systemic hypertension. Although hypothesis generating, the study highlighted the link between hemodynamic stresses, atrial structural remodeling and arrhythmogenesis.

4. Endothelin axis and clinical arrhythmogenesis

With the importance of the Endothelin system in tissue structural remodeling and fibrosis in addition to its vaso-modulatory properties increasingly recognized, studies have looked into its relationship with clinical arrhythmogenesis. This has led to several epidemiological studies into its influence on outcomes in clinical AF. Investigators from the GISSI-Atrial Fibrillation study into the efficacy of valasartan in AF recurrence performed a circulating biomarker sub-study looking at baseline and follow-up levels of cardiac specific and vasoactive peptides and their relationship to AF recurrence following cardioversion\textsuperscript{180}. Despite low levels at baseline, elevated Pro-Endothelin-1 (CT-Pro-ET-1) was significantly predictive of arrhythmia recurrence in multivariate modeling. However, given that the overwhelming majority of patients enrolled in this study were hypertensive, there authors recognized a potential intrinsic bias thus limiting the interpretation of these findings. This may be particularly important when evaluating a possible pathophysiologic role of ET-1 in view of its potent arterial vasoconstriction
properties. To further elucidate the role of this system in AF, investigators studied protein and mRNA levels of ET-1 and its receptors ET-A and ET-B in right atrial appendage tissue from patients with paroxysmal and persistent AF in both the presence and absence of valvular disease\textsuperscript{181}. Levels of pro-ET-1 mRNA were increased in patients with valvular heart disease, whilst receptor protein levels were reduced in paroxysmal and persistent AF patients regardless of the presence of valvular heart disease. Furthermore, mRNA markers of receptor expression were only abnormal for ET-B in patients with persistent, but not paroxysmal AF. These findings provide evidence for distinct roles of ET-A and ET-B in different phenotypes/models of AF and the role of the endothelin axis in disease progression. The significance of the Endothelin system in AF therapeutic outcomes was further studied in patients undergoing pulmonary vein isolation\textsuperscript{182}. Patients without structural heart disease were followed up 6 months post ablation. Serum natriuretic peptides, angiotensin II, aldosterone, renin and ET-1 were measured. On multivariable modeling, only ET-1 was predictive for procedural failure and early recurrence. Furthermore, there was a significant correlation between serum ET-1 and LA pressure. Taken together, circumstantial evidence and preclinical work implicate ET-1 as an important mediator of both atrial remodeling and atrial arrhythmogenesis.

**Atrial dilation: macro-structural remodeling**

Although many studies have provided insights into the atrial remodeling process in various conditions and proposed ways by which these changes may predispose to AF
development and sustenance, the clinical correlate has been more limited. Clinical assessment of LA health has been limited to size (area and volume), however recent advances in Magnetic Resonance Imaging MRI technology has seen the emergence of non-invasive atrial scar characterization as a viable prognostic tool for AF disease progression and outcomes following catheter ablation. Oakes et al. used delayed enhancement MRI to characterize atrial scar burden and correlate this with electro-anatomical voltage mapping\textsuperscript{183}. There was a direct correlation between the burden of atrial scar and the rate of arrhythmia recurrence following pulmonary vein antral isolation. This study was the first to provide a clinical tool by which atrial scar can be assessed non-invasively. However, the study was of relatively short duration and the burden of scar was arguably author designated (mild, moderate and severe), the clinical significance of which remains unclear. Furthermore, the technologies employed in this investigative setting remain limited in their wider clinical applicability at this point. Therefore, more conventional means of assessing LA size, by MRI and transthoracic echocardiography, currently remain popular. It is generally agreed that echocardiographic LA volume, rather than area\textsuperscript{184}, and that assessment by MRI may represent the gold standard\textsuperscript{185}. Echocardiographic volumetric assessment is often performed by either the modified Simpson method or the area-length method. To adjust LA size to \textit{body size}, indexing is often performed. It is unclear if indexing to body surface area attenuates a clinically prognostic meaningful relationship between chamber size and adiposity\textsuperscript{20,186}. It is suggested that ideal indexing (to account for body size) should be made to height only, rather than body surface area (which may falsely
attenuate clinically important obesity-related atrial dilation estimates). The LA volume appears to act as a barometer of the underlying pressure of volume stress, resulting in an elevated filling pressure. In addition, LA volume provides long term prognostic information on mortality and AF development in ischemic and non-ischemic cardiomyopathy patients, as well as ambulatory outpatient clinic populations.\textsuperscript{187, 188}

Whilst LA volume is believed to reflect left ventricular diastolic function, other factors are known to be associated with LA dilation. These may represent either mechanistically independent predictors or conditions which may mediate atrial dilation through diastolic dysfunction. To examine this effect, Otto and colleagues conducted a small population study comparing the LA size (volume indexed to height) in obese healthy patients and obese patients with co-morbid OSA.\textsuperscript{189} The latter group had a significantly larger indexed LA volume, than the former group. Importantly, the OSA group did not have AF, therefore, suggesting an obesity-independent relationship between OSA and atrial remodeling. Although the groups were matched with regards to systolic and diastolic blood pressure, it was unexpectedly ‘normal’ in both groups (OSA group 128/76, non-OSA group 126/79), which may suggest an underrepresentation of hypertension (a common disease in both obesity and OSA) in a select group. This relationship between hypertension and LA dilation was studied by Gerdts et al.\textsuperscript{190} In a combined echocardiographic and electrocardiographic observational study of hypertensive subjects, LA size dilation was associated with gender, age, obesity, systolic blood pressure and eccentric left ventricular hypertrophy (LVH) and was independent of AF, left ventricular mass and the presence of mitral regurgitation. Although illustrating
the relationship between LA remodeling and systolic blood pressure, the investigators sub selected a hypertensive population with electrocardiographic LVH. Generally, electrocardiographic features of LVH are limited in predicting the presence of structural hypertrophy on imaging.

The relationship between coronary epicardial disease and LA dilation has been previously studied. Boyd et al. underwent echocardiographic assessment of LA volumes immediately (48 hours) following an acute non-ST elevation coronary syndrome, and re-imaged at 12 months\textsuperscript{191}. The group demonstrated a progressive increase in volume with the presence of epicardial major coronary disease and DM as predictors of LA enlargement. This relationship between coronary disease and atrial remodeling was also demonstrated by Pan et al.\textsuperscript{192}. Using coronary calcium scores on multi-detector computed tomographic imaging, the investigators showed that a higher coronary calcium burden was associated with greater LA diameters, appendage orifice size and pulmonary vein orifice size. Although atrial volume was not assessed in this study, it nevertheless alludes to the association between coronary arterial disease and atrial remodeling, perhaps predisposing to AF.

Various studies have demonstrated an adverse relationship between obesity and LA size, as a surrogate for LA remodeling. Stritzke et al. conducted a 10 year prospective study in a large mixed gender population\textsuperscript{20}. The investigators used LA volume indexed to height in their calculations. The findings showed that hypertension and obesity being independent predictors of LA enlargement, with obesity being the statistically more
robust predictor. The highest indexed volume was found in the combined hypertensive obese population. More recently, munger et al. directly measured LA pressure in obese patients and demonstrated a significant elevation relative to normal weight patients\textsuperscript{193}. It is however worth noting that the 2 groups were not matched for the presence of DM, OSA and hypertension. Therefore, on the weight of the evidence, there appears to be a relationship between obesity and LA dilation, and this relationship may be independent of various obesity associated co-morbidities.

**Obesity and therapeutic outcomes of atrial fibrillation**

Although obesity is perceived to promote a worse AF disease progression and severity, data regarding therapeutic outcomes remain preliminary and confounded by other obesity-related co-morbidities. Moreover, most studies have focused on the outcomes pertinent to catheter-based ablation. Jongnarahsin et al. compared outcomes of a single ablation procedure in patients across the 3 BMI categories\textsuperscript{194}. There were 63% of patients free of AF in the group without OSA, whereas 41% were free of AF in the group with OSA, after a mean follow-up period of 7 months, suggesting a detrimental effect of OSA on ablation outcomes. On multivariate analysis, the adverse effect of OSA was sustained despite adjusting for type and duration of AF, BMI, LA size, LV ejection fraction and the presence of hypertension. This suggested that perhaps OSA was a more potent predictor of early procedural failure as compared to the obesity, LA dilation and hypertension. Chilukuri et al. on the other hand presented a longer follow-up of 11 months\textsuperscript{195}. Again, OSA was a poor prognostic factor, however on multivariate analysis
only BMI remained predictive of procedural failure. It is critical however to note that
OSA was diagnosed using the more comprehensive polysomnography study in the
former study, whereas the latter study utilized the screening Berlin questionnaire tool,
which may have a more limited applicability in this high risk cohort of patients. An
interesting consideration when determining the risk-benefit ratio for catheter ablation
of AF in obese patients is radiation dose. This has become a major focal issue recently.
Ector calculated the radiation dose in 85 patients undergoing catheter ablation and
stratified patients according to weight class\textsuperscript{196}. The obese group was shown to receive
the highest radiation dose of all three weight groups, interpreting to an approximate 2.5%
lifetime risk of malignancy, compared to normal weight patients. However, despite
these discouraging indicators, Cha et al. conducted a quality of life based assessment of
ablation outcomes. Although weight was shown to adversely affect freedom from AF
rates, the quality of life, assessed by SF-36 scores, improved in all BMI categories.
Notably, despite the obese group having the ‘worst’ quality of life when AF is co-existent,
freedom from AF was associated with a significant improvement in SF-36 scores,
suggesting perhaps that this is the very group that derives the most benefit from an
invasive procedure. This was again confirmed in a study by Mohanty utilizing more
detailed quality of life questionnaires\textsuperscript{197}. The group showed that although
overweight/obese patients had worse baseline scores, post successful ablation they
derived the greatest benefit by having the largest increase in scores as compared to
normal weight counterparts. Furthermore, beyond obesity alone, these results were
applicable to patients with the full metabolic syndrome spectrum (including those with
elevated systemic inflammatory markers at baseline\textsuperscript{198}. Specifically, elevated levels of high sensitivity C-reactive protein, a phenomenon often observed in obesity and the metabolic syndrome has been shown, by Liu et al. in a meta-analysis, to predict post successful electrical cardioversion relapse\textsuperscript{199}. Finally, no study has identified obesity as a predictor of increased peri-procedural complications.

**Role of risk factor modification**

Cardio-metabolic risk factors often coexist with AF and have been regarded as major risk factors for the arrhythmia. These conditions often overlap or congregate in patient syndromes (eg. hypertension-sleep apnea, “metabolic syndrome”), thus making cause and effect difficult to elucidate. Nevertheless, with obesity being identified as the epidemiological driver behind these conditions, its presence therefore demands assessment, identification and management.

**Obesity and weight management**

1. **Overview of barriers to weight loss and lifestyle modification**

It is widely considered that weight gain is a result of a chronic imbalance between levels of energy intake and energy expenditure. Therefore the cornerstone of most weight loss treatments has been to reverse this imbalance through caloric restriction and promoting a lifestyle conducive to greater activity levels. In the current obesogenic environment, this has posed enormous challenges resulting in an epidemic which pervades well into most age groups and demographics.
Several issues currently remain unanswered when considering the obesity dilemma at the level of the clinical patient encounter. Firstly, the optimal psycho-cognitive method to elicit behavioral change and delivery of the weight loss program, remain unclear. Secondly, the macronutrient composition and proportion for a general population-applicable diet conducive to weight management, remains unknown. Thirdly, and perhaps the most profound of all challenges is the ability to sustain the behavioral change and long term benefits of weight maintenance following successful initial weight reduction. Another related uncertainty is the extent of benefit of various lipid micro-nutrients (marine and plant mono and poly unsaturated triglycerides) in promoting weight management and providing a favorable cardio-metabolic profile with regards to low-grade inflammation, lipid sub-fractions and blood pressure management. This is critical in view of the intricate relationship between dietary constituents, BMI and systemic arterial blood pressure.

2. Causes and progress of obesity in adults

“Overweight and obesity are multi factorial conditions with genetic and environmental elements” is a commonly resonated statement, reflecting both the far from incomplete understanding of their pathogenesis and management difficulties. Studies have alluded to a metabolic equilibrium set point towards which weight will re-converge towards over time, following an initial divergence (so-called metabolic set-point theory). It is plausible that intra uterine and early childhood factors such as birth weight, maternal nutrition, maternal smoking, maternal diabetes, breast feeding and childhood sedentary
lifestyle may play a role in setting this equilibrium point. Environmental factors critical in the pathogenesis of obesity are beyond the availability of refined calorie-dense readily available foods. Hu et al. illustrated in the Nurses’ health study that amongst all sedentary lifestyle factors, a strong dose-dependent relationship existed between time spent watching television and levels of obesity.

Other environmental factors include, pattern of eating, serving sizes, sleep patterns and interestingly, the social network of the patient. The latter was demonstrated using longitudinal statistical methods to analyze the pattern of subject-to-subject ‘spread’ of obesity, by Christakis et al., akin to an infectious disease outbreak.

**Overview of dietary therapeutic strategies for obesity management in adults**

The strategic therapeutic paradigm for the management of the diagnosed obese adult is dietary restriction and exercise, within the context of a permanent behavioral modification program.

The purpose of behavioral modification is to equip patients with techniques to initiate weight loss and sustain weight lost through; developing a better relationship with food and nutritional insight, developing realistic long-term goals, integrating physical activity into their lifestyle and recognizing cues and triggers that stimulate unhealthy eating patterns. It appears that the success of any program that incorporates these features must utilize frequent patient contact, either remote or face-to-face. It is plausible that
this strategy fosters a degree of accountability for the incremental goals set. Digenio et al. conducted a randomized controlled study looking at frequency (high or low) of contact and the method of communication (telephone, email, face to face) or “self-directed”. Although the study was limited to females with a relatively short follow-up period of 6 months, the frequent contact (either face to face or telephone) was more superior in terms of quality of life and absolute weight reduction. This was confirmed by Appel et al. with longer follow-up and a more heterogeneous patient population. Delivered either remotely or face to face, the weight loss program resulted in more superior weight reduction relative to a self-directed weight reduction group.

*Dietary therapy for obesity management*

When constructing incremental goals for weight loss, it is critical to take gender and age into account so as to be able to set realistic goals. Furthermore, it is useful to pre-empt the dynamics of weight loss with the expectation of a plateau during the trajectory, as a form of adaptation. It may therefore be useful to develop a primary accelerated weight loss phase followed by a slower secondary phase of sustained weight loss focusing on weight maintenance through lifestyle change. There are several generic dietary methods to achieve weight control or weight loss.

1. **Very low calorie diet (VLCD)**

The VLCD limits energy intake to 800 calories per day, with inclusion of all necessary micronutrients. This method is effective in achieving a rapid result weight loss, this
providing a psychological reward for the patient. The VLCD has been shown to promote an insulin sensitizing effect in the obese diabetic\textsuperscript{209}. In addition, this method induces a hypotensive effect, which may necessitate anti-hypertensive management initially. Tsai et al. has demonstrated in a meta-analysis that VLCD programs are superior to low calorie diets for inducing an initial effective weight reduction, however sustained differences were ameliorated with time\textsuperscript{210}.

2. **High protein diet**

The high protein diet is also effective for weight reduction and may have a favorable effect on cardiovascular inflammatory markers\textsuperscript{211}. Larsen et al. showed favorable weight loss sustenance (less weight re-gain) on a diet higher in protein and lower in high glycemic index foods\textsuperscript{212}. The short-term effectiveness of high protein diets was also demonstrated by Claessens et al.\textsuperscript{213}. It may therefore be reasonable to conclude that, although high protein diets are effective in weight reduction, their sustainability may be difficult to implement. Moreover, their long-term safety remains untested rigorously.

3. **Low carbohydrate diet**

Proponents of low carbohydrate diets suggest that nutrient poor, calorie dense foods are the most obesogenic factors in our environment. Nordmann conducted a meta-analysis on low carbohydrate diets and concluded that these strategies are effective in the short term, but not beyond 12 months\textsuperscript{214}. Moreover, the beneficial effects on the lipid profile (increased HDL and decreased triglycerides) need to be weighed against the
detrimental effects of increasing LDL. For the latter, a low fat diet may be the most efficacious. Finally, the low carbohydrate diet may provide a false sense of successful weight loss. The initial effect of such strategy is the induction of glycogenolysis and mobilization of skeletal glycogen stores. Being hydrophilic, this causes a diuresis, which results in fluid loss rather than an actual change in abdominal adiposity. This consideration needs to be made when initiating this form of diet.

4. Low fat diet and portion controlled diet

In contrast to the above strategies, a low fat diet has been shown to have a maintaining effect following weight loss, as illustrated in the Women’s Health Initiative Study by Howard et al.\textsuperscript{215}. Similarly, portion controlled meal plans (either frozen pre-prepared meals or liquid meal replacements) may be effective in long term weight maintenance, following weight reduction\textsuperscript{216}.

5. Mediterranean diet

The salient features of this form of diet is the inclusion of moderate alcohol (wine) consumption, dairy (cheese), legumes, grains, fruits, vegetables, higher monounsaturated fat intake and low red meat intake. This dietary strategy may be more attractive as it is seen as less restrictive. Furthermore, in a meta-analysis of 8 studies by Sofi et al., the benefits of a Mediterranean diet included a reduction in the risk of malignancy, cardiovascular disease, neurodegenerative diseases and overall mortality\textsuperscript{217, 218}. 
Notwithstanding the advantages and disadvantages of the each of the above strategies, it is generally accepted that the key to successful weight loss and weight maintenance is adherence to the patient-specific dietary program utilized, its combination with a graduated sustainable regular exercise plan and the delivery of the program through regular reinforcement that engenders patient empowerment and accountability\textsuperscript{219}. However, equally, the physician must be accountable for this difficult responsibility.
Title of Thesis:

Obesity and Atrial Electrical and Mechanical Remodeling: Implications for Atrial Fibrillation.

Name:

Dr. Hany S. Abed

Chapter 2

Obesity results in progressive atrial structural and electrical remodeling:

Implications for atrial fibrillation

Short Title: Abed, et al. Obesity & Atrial Fibrillation

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Keywords: Atrial fibrillation, remodeling, conduction velocity, obesity, fibrosis

Abbreviations:

AF – Atrial fibrillation

CCF – Congestive cardiac failure
IQR – Inter-quartile range

LA – Left atrium

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**ABSTRACT**

**Background**

Obesity is associated with atrial fibrillation (AF); however, the mechanisms by which it induces AF are unknown.

**Objectives**

To examine the effect of progressive weight gain on the substrate for AF.

**Methods**

Thirty sheep were studied at baseline, 4 months and 8 months, following a calorie-dense diet. Ten sheep were sampled at each time point for cardiac magnetic resonance imaging (CMR) and hemodynamic studies. High density multi-site bi-atrial epicardial mapping was used to quantify effective refractory period (ERP), conduction velocity (CV) and conduction heterogeneity index (CHI) at 4 pacing cycle lengths (CL) and AF inducibility. Histology was performed for atrial fibrosis, inflammation, intramyocardial lipidosis and molecular analysis performed for Endothelin-A and -B (ET_{A/B}) receptors, Endothelin-1 (ET-1) peptide, platelet derived growth factor (PDGF-BB), transforming growth factor (TGF-β1) and connective tissue growth factor (CTGF).

**Results**

Increasing weight was associated with increasing left atrial (LA) volume (P=0.01), fibrosis (P=0.02), inflammatory infiltrates (P=0.01) and lipidosis (P=0.02). While there was no change in ERP (P=0.2), there was slowing in CV (P<0.001), increased CHI (P<0.001) and increased inducible (P=0.001) and spontaneous (P=0.001) AF. There was increased atrial cardiomyocyte ET_{A/B} receptors (P=0.001) and ET-1 (P=0.03) with increasing adiposity. In
association, there was a significant increase in atrial interstitial and cytoplasmic TGF-β1 (P=0.02) and PDGF-BB (P=0.02) levels.

Conclusion

Obesity is associated with atrial electro-structural remodeling. With progressive obesity there were changes in atrial size, conduction, histology and expression of pro-fibrotic mediators. These changes were associated with spontaneous and more persistent AF.

Key Words

Obesity, Atrial fibrillation, Remodeling, Fibrosis, Conduction
INTRODUCTION

Obesity is recognized to be associated with the development of atrial fibrillation and has been proposed as a contributor to the expanding epidemic of this arrhythmia \(^7,14\). Atrial structural and electrical remodeling have been implicated in the AF substrate associated with many conditions predisposing to the development of this arrhythmia \(^28,60,63,104\); however, whether weight gain and obesity result in atrial remodeling is not known. Moreover, induction of this substrate along the adiposity spectrum of normal weight to obesity, and its relationship to the hemodynamic disturbances, remains unknown. In this study, utilizing a sheep model of progressive weight gain, we aimed to characterize the atrial functional, structural and electrophysiological changes accompanying increasing adiposity.

METHODS

Animals

Thirty-six sheep (Merino-cross Wethers) were studied in accordance with guidelines outlined in the “Position of the American Heart Association on Research Animal Use”, adopted November 11 1984. This study was approved by the Animal Ethics Committees of the University of Adelaide and SA Pathology, Adelaide, Australia.

Study Protocol

Thirty animals underwent ad-libitum feeding to induce obesity, as previously described \(^220\). At baseline, 4 months and 8 months, 10 of the cohort were randomly
selected for CMR followed by open chest electrophysiology study. An additional 6 sheep were studied (3 at each of the 2 time-points; 4 months and 8 months) as controls.

**Ad-Libitum Feeding Obese Ovine Model**

A previously characterized model of progressive weight gain, utilizing an ad-libitum regimen of hay and high energy pellets, was used to induce progressive weight gain\(^ {220} \). This model showed an approximate increase of 10kg monthly up to 8 months following which weight gain reached a plateau. In brief, at baseline, 30 healthy animals were commenced on a high caloric diet of unlimited supply of energy-dense soybean oil (2.2%) and molasses fortified grain and maintenance hay with weekly weight measurement. Excess voluntary intake was predominantly of grass alfalfa silage and hay. For the obese sheep, pellets were gradually introduced at 8% excess basal energy requirements, and rationed to \( \geq 70\% \) of total dry matter intake. Blood samples were periodically collected to ensure electrolyte, glucose and acid-base homeostasis. To maintain the 6 controls at their baseline weight, hay was distributed for maintenance, while energy dense pellets were rationed at 0.75% of live weight daily to maintain weight tightly between 50-60kg. Nutritional content of food and housing conditions were identical between both groups, but only the amount was varied. Shorn weight was recorded immediately prior to surgery. Study outline is illustrated in (Figure1).

**Cardiac Magnetic Resonance Imaging**
Chamber volumes were measured using CMR (Siemens Sonata 1.5 Telsa, MR Imaging Systems, Siemens Medical Solutions, Erlangen Germany) with 6mm slices through the atria and 10mm through the ventricles without inter-slice gaps. Animals were securely placed in the dorsal recumbent position for scanning. Mechanical ventilation was maintained, facilitating ECG-gated image acquisition with periodic breath holding. Analyses were performed offline by blinded operators using proprietary software, QMass MR (Medis medical imaging systems, Leiden, Netherlands). Chamber size, ventricular mass and pericardial fat volumes were measured using previously described methods.\textsuperscript{93}

**Animal anesthesia**

Intravenous sodium thiopentone (15-20mg/kg) was used for induction before endotracheal intubation. Isoflurane in oxygen (2-4%) was used for maintenance. Invasive arterial blood pressure, heart rate, pulse oximetry, end-tidal CO\(_2\) and temperature were monitored continuously.

**Hemodynamic Recordings**

Continuous invasive mean arterial pressure (MAP) monitoring was undertaken at the time of electrophysiology study. In addition, direct LA catheterization was performed to measure LA pressure (LAP).
**Electrophysiology Study**

Electrophysiology study was performed under general anesthesia. Midline sternotomy was used to facilitate pericardial cradle formation and epicardial application of custom designed 128-electrode plaques with 5mm spacing, spanning the appendage and free wall on each atrial chamber, as previously described\textsuperscript{28}. Plaques were connected to a computerized signal digital analyzer (LabSystem Pro, Bard Electrophysiology, Lowell MA). Surface ECG and epicardial electrograms were recorded for offline analysis. All electrograms were filtered between 30-500Hz and measured with computerized calipers at a sweep speed of 200mm/sec.

**Atrial Effective Refractory Period (ERP)**

Atrial ERP was measured at twice the tissue capture threshold at CL (S1) of 500, 400, 300 and 200ms from four sites (right atrial appendage [RAA], right atrial free wall [RAFW], left atrial appendage [LAA] and left atrial free wall [LAFW]). Eight basic (S1) stimuli were followed by a premature (S2) stimulus in 10ms decrements. Atrial ERP was defined as the longest S1-S2 interval not resulting in a propagated response. Each measurement was repeated three times. If there was greater than 10ms variability, two further measurements were taken and the total averaged.

**Atrial Conduction Velocity (CV)**

Conduction velocity was calculated from each site, during stable capture of S1 pacing train and the shortest coupled S2 that captures the atria at each CL. Activation maps
were created using semi-automated custom software, as previously described\textsuperscript{28}. Each annotation was manually verified with local activation timing annotated to the peak of the largest amplitude deflection on bipolar electrograms. Local conduction velocity was calculated from the local vectors within each triangle of electrodes. Mean CV was then derived for each map.

\textit{Atrial Conduction Heterogeneity}

Conduction heterogeneity was assessed using established phase mapping techniques during S1 pacing\textsuperscript{28,63}. In brief, the largest activation time difference between every four adjacent electrodes was first determined and divided by inter-electrode distances. The largest value at each site was then used to create a phase map, with values also displayed as histograms. Absolute conduction phase delay was calculated as the difference between the 5\textsuperscript{th} and 95\textsuperscript{th} percentile of the phase difference distribution. Conduction heterogeneity index is then determined by dividing the absolute phase delay by the median ($P_{50}$).

\textit{AF Induction}

Spontaneous and induced AF episodes were documented during continuous recording for the duration of the study with off-line verification. Spontaneous AF was defined as episodes of irregular atrial rhythm lasting ≥5sec occurring during the electrophysiology study in the absence of pacing after stable plaque application. Inducibility of AF was determined by ramp burst pacing commencing at CL 250msec with progressive 5ms
decrements until AF was induced or there was no longer 1:1 capture of the atria (to atrial refractoriness). This maneuver was repeated 5 times from each of the 4 per-determined pacing sites and undertaken after the completion of electrophysiologic evaluation. When AF was induced and persisted for ≥5sec, the episode and its duration was recorded. Electrical cardioversion was performed only when hemodynamic compromise occurred or if AF became sustained (≥5mins). If cardioversion was required or arrhythmia sustained, no further electrophysiologic recordings were made.

**Structural Analysis**

At study completion, the heart was removed. From each animal, 6 sections were harvested from the LA, LAA, RA and RAA, and fixed in 10% buffered formalin or frozen at -70°C. Quantitative analysis of wax-embedded specimens for percentage collagen was performed using Picrosirius red staining. Viable sections from each atrium of every animal were digitally captured (20 sections/animal) with an area of picrosirius red selected for its color range and the proportional area of tissue with this range of color quantified.

For lipid content analysis, 4 sections of frozen myocardium from each animal were air dried and formalin fixed, after careful epicardial fat stripping. Sections 5-7μm thick were prepared with Oil-red-O 0.3% w/v isopropanol and distilled water for 15 minutes then washed with 60%isopropanol. Five random non-overlapping fields were captured and digitized using a Carl-Zeiss microscope attached to AxioCamMRC5 digital camera (Carl-
Zeiss, North Ryde, Australia) at X200 magnification. An area of red (lipid) was selected for its color range and the proportional area of tissue with this range of color then digitally quantified and expressed as proportional per area. Cellular infiltrates were visualized using hematoxylin and eosin (H&E) staining and graded using a previously described scale of 0-2\(^{221}\). All analyses were performed blinded.

**Pro-fibrotic molecular markers**

**Western blotting**

Western blotting was used to assess changes in ET receptor expression in atrial myocardium between weight groups. Total protein was extracted from the atria as described before and quantified by the Bradford protein assay. To determine changes in Endothelin (ET) receptor expression between groups, protein extracts (containing an equal amount of 10-15\(\mu\)g of total protein/lane) were electrophoresed under reducing conditions on 10.5% acrylamide gels. Western blot analyses were performed with polyclonal antibodies to the ET\(_A\) receptor (ab30536; 1:500 dilution; Abcam, Cambridge, MA, USA) and ET\(_B\) receptor (AF4496; 1:300 dilution; R&D Systems, Minneapolis, MN, USA), in addition to a goat anti-sheep secondary antibody. A monoclonal antibody to the house keeping protein, \(\alpha\)-tubulin (1:8000 dilution; Millipore Corp., Billerica, MA, USA) was used to demonstrate equivalent loading of protein samples. Densitometry of ET\(_A\) (63kDa) and ET\(_B\) (\(\sim\)30kDa) receptor bands was performed using Bio-Rad GS710 Calibrated Imaging Densitometer and Quantity-One\(^{TM}\) software (Bio-Rad Laboratories,
The density of each receptor was then corrected for corresponding α-tubulin and expressed as an absolute optical density (OD, arbitrary units).

Immunohistochemistry

The pro-fibrotic and proliferative effect of ET signaling is mediated by a variety of putative molecules. In order to determine which factors contributed to the obesity-induced atrial fibrosis observed, the expression and distribution of transforming growth factor (TGF)-β1, connective tissue growth factor (CTGF), platelet-derived growth factor (PDGF-BB), endothelin-1 (ET-1), ET<sub>A</sub> and ET<sub>B</sub> in the 3 groups, was determined by immunohistochemistry. Serial sections from paraffin-embedded atrial tissues were stained using polyclonal antibodies to i) TGF-β1 (sc-146; 1:200 dilution; Santa Cruz Biotechnology; Santa Cruz, CA, USA); ii) CTGF (ab6992; 1:400 dilution; Abcam; Cambridge, MA, USA); iii) PDGF-BB (ab21234; 1:50 dilution; Abcam); iv) ET<sub>A</sub> (ab30536; 1:100 dilution; Abcam); and v) ET<sub>B</sub> (ab50658; 1:100 dilution; Abcam); or vi) a monoclonal antibody to ET-1 (E166; 1:1000 dilution; Sigma Aldrich, St Louis, MO, USA). Non-specific protein binding and endogenous peroxidase were blocked by incubating sections with antibody diluent containing protein (DAKO, Carpintaria, CA, USA) and 3% H<sub>2</sub>O<sub>2</sub>, respectively, while antigen retrieval was performed by heating sections in citrate buffer pH6.0. All primary antibodies were incubated overnight with the exception of TGFβ1, which was incubated for 4 hours. Polyclonal primary antibodies to TGF-β1, CTGF and PDGF were detected with the EnVision anti-rabbit kit (DAKO); the monoclonal antibody
to ET-1 was detected with the EnVision anti-mouse (DAKO); and polyclonal antibodies to ET\textsubscript{A} and ET\textsubscript{B} were detected with an anti-sheep secondary IgG antibody (DAKO) absorbed with normal sheep serum. Sites of bound antibody were identified using 3,3-diaminobenzidine (DAKO) and sections counterstained with hematoxylin. Negative controls consisted of omission of the primary antibody. Morphometric analysis was performed using ImageJ 1.3 software (National Institutes of Health, Bethesda, MD, USA); from 5-8 random fields from each section analyzed per sample and per group. In each case, the percentage staining of each marker analyzed per field was derived.

**Statistical Analysis**

Continuous variables are expressed as mean±SD. Hemodynamic variables, histologic indices and AF episode number/duration are expressed as median and IQR with differences determined using the Kruskal-Wallis test. The Mann-Whitney U test was used for pair-wise comparisons. One-way ANOVA was used to determine differences in CMR measures across weight cohorts. Pearson’s correlation coefficient was used to determine the relationship between weight and AF episodes. The latter was log transformed due to its non-normal distribution. To investigate the effect of progressive obesity on electrophysiological parameters, a linear mixed-effects model was used with each electrophysiological parameter (ERP, CV and CHI) entered as the dependent variable to determine the effects of weight cohort, for both S1 and S2-derived recordings. Fixed factors of weight cohort, CL and site (appendage and free wall of each
corresponding atrium) were entered for main effects. Animal ID was entered as a random factor to account for nested data within each experiment. Bonferroni method was use for pairwise comparisons of continuous variables. Hemodynamic variables (MAP and LAP) were entered as covariates. To determine any possible time-related changes in electrophysiology, baseline, 4 month and 8 month time-point controls were similarly entered into a linear mixed-effects model. Variables were log transformed to satisfy model assumptions. Statistical significance was established at P<0.05. All analyses were performed using SPSS version18 (SPSS, IBM).

RESULTS

There was progressive weight gain with feeding duration from 58±7kg at baseline to 77±5kg (‘overweight’) at 4 months and 105±13kg (‘obese’) at 8 months (P<0.001). There was no weight change in the control group; 58±6kg at baseline, 50±4kg at 4 months and 54±5kg at 8 months (P=0.2). Electrolyte, acid-base and glucose levels remained within normal range throughout the overfeeding process.

Functional and Structural Changes

Twenty-two CMR scans were available for analysis. The remaining 8 were excluded due to artifact. Figure 2 shows a significant progressive increase in LA (P=0.01) and RA (P=0.04) volumes with increasing weight gain and likewise, a progressive increase in bi-ventricular myocardial mass (P<0.001) and pericardial fat volume (P<0.001). There were no significant differences in ventricular volumes or function. All parameters in the
control group remained stable. Interclass correlation coefficient between 2 independent observers for LA and RA volumes were 0.87 and 0.92, respectively. For other CMR measures, intra-observer and inter-observer coefficients of variation were 3.5% and 4.9%, respectively. There was a graded increase in MAP and LAP (P=0.02 and P<0.001 respectively), with increasing weight gain.

**Atrial Electrophysiology**

*Effective Refractory Period*

With increasing adiposity there were no significant differences in ERP between groups at any site; LAA P=0.2, LAFW P=0.8, RAA P=0.08, RAFW P=0.2. Likewise, there was a non-significant increase in ERP between baseline, 4 month and 8 month controls (LAA: baseline: 195±10 msec, overweight: 216±18 msec, obese: 217±18, P=0.4).

*Atrial Conduction*

**Figure 3A** contains the changes in CV with increasing weight. **Figure 4A** shows the progressive conduction slowing demonstrated by isochronal crowding and delayed activation. There was progressive conduction slowing with increasing weight, most profound in the obese group; LAA P<0.001, LAFW P=0.001, RAA P=0.001, RAFW P=0.001. Adjusting for hemodynamic changes, conduction slowing remained significant; LAA P=0.01, LAFW P=0.01, RAA P=0.01, RAFW P=0.03. While baseline and overweight animals demonstrated no significant differences in CVs between CLs, obese animals demonstrated marked CL-dependency of CV with significantly greater slowing at shorter
CLs; conduction slowing was significantly greater at CL 200ms compared to CL 500ms only in the obese cohort; RAFW P=0.03, RAA P=0.004, LAFW P=0.04, LAA p=ns. Extending these observations, Figure 4B demonstrates the differences in CV at baseline, overweight and obese animals in terms of S1 and S2. As expected, the S2 coupling interval demonstrated slower conduction. With increasing obesity, the extent of the conduction slowing with S2 compared to S1 was greater (P<0.001). These features suggest a functional component to conduction slowing as a result of obesity and illustrate the impact of premature beats on the underlying substrate.

Associated with atrial conduction slowing was an increase in regional conduction heterogeneity, with increasing adiposity (Figure 3B). There was an increase in CHI with progressive weight gain. At slower pacing (CL 500ms), while CHI was progressive with increasing weight; LAA P=0.008, LAFW P=0.001, RAA P=0.001, RAFW P<0.001, after adjustment for hemodynamic variables (MAP and LAP), at some sites this relationship between obesity and CHI was weakened; LAA P=0.3, LAFW P=0.2, RAA P=0.1, RAFW P=0.003. However, with faster pacing (CL 200ms) there was a global increase in heterogeneous conduction (P<0.05 for all sites); this effect largely persisted despite statistical adjustment for hemodynamic variables; LAA P=0.2, LAFW P=0.03, RAA P=0.02, RAFW P=0.001.

In the control cohort, there were no significant changes (either site or CL) in CV (m/sec) (LAA: Baseline 1.05±0.02, 4 months 0.99±0.04, 8 months 1.04±0.04; P=0.5) and CHI (LAA: Baseline 1.17±0.04, 4 months 1.17±0.07, 8 months 1.19±0.07; P=0.96).
**AF Burden**

**Figure 5** shows the AF burden, quantified by number of episodes (spontaneous and induced) and duration of arrhythmia. There was a significant association between weight and an increase in spontaneous episodes (P<0.001), inducible episodes (P=0.001) and cumulative duration of AF (P=0.01).

**Fibrosis, Cellular Infiltrates and Lipidosis**

**Figure 6** demonstrates representative examples of atrial histology using Picrosirius, H&E and Oil-red-O staining for animals at baseline, overweight and obese. Atrial tissue demonstrated distorted myocyte arrangement and widening of the interstitium with obesity. Quantitative histology showed increased perivascular collagen deposition with increasing adiposity, greatest in the obese group (LA P=0.02, RA P=0.01). Myocardial lipidosis occurred early in the overweight group and progressively increased (LA P=0.02, RA P=0.07). On H&E staining, with increasing weight there was increasing interstitial inflammatory cellular infiltrates in atrial tissue (LA P=0.01, RA P=0.05).

**Pro-fibrotic markers**

On Western blot there was an increase in ET\textsubscript{A} (P=0.001) and ET\textsubscript{B} (P=0.001) receptor levels in atrial tissue (**Figure 7A**) in the overweight and obese groups relative to baseline, which for both receptor subtypes peaked in the overweight group. Immunostaining, at the cardiomyocyte plasma membrane for both receptor protein subtypes, was strongest in the obese and overweight groups, relative to the baseline group.
(P=0.001 for ET\textsubscript{A} and ET\textsubscript{B}) \textbf{(Figure 7B)}. Staining for cytoplasmic ET-1 ligand increased modestly in the obese group, relative to baseline (P=0.03). There was a significant correlation between increasing weight and atrial interstitial and cytoplasmic PDGF-BB \textbf{(Figure 8)}; the highest expression was seen in the obese cohort (P=0.02). There was a significant rise in cytoplasmic CTGF with early weight gain (P=0.03), followed by a non-significant reduction in the obese cohort. There was a significant correlation between weight and atrial interstitial and cytoplasmic TGF-\(\beta\)1 (P=0.02).

**DISCUSSION**

**Major Findings**

Progressive weight gain resulted in atrial functional, structural and electrophysiological remodeling characterized by:

1. Increased atrial volumes, LA and systemic pressures, ventricular mass and pericardial fat volumes;
2. Increased atrial interstitial fibrosis, inflammation and myocardial lipidosis;
3. Progressive conduction abnormalities with slowing of atrial conduction and increased conduction heterogeneity, which was amplified at shorter coupling intervals and CLs with greater adiposity. The significance of these abnormalities with progressive adiposity persisted after adjusting for potential hemodynamic variables. There was no change in tissue ERP.
4. Over-expression of atrial cardiomyocyte ET\textsubscript{A/B} receptors and a weak association with cytoplasmic ET-1 levels;
5. Increase in interstitial and cytoplasmic TGF-β1, PDGF-BB, and CTGF levels;
Perhaps as a consequence of these abnormalities, progressive weight gain was associated with a greater burden of induced and spontaneous AF.

**Atrial Remodeling and the Substrate for AF**

Li and Nattel in a rapid ventricular pacing model of heart failure provided the seminal observations of the importance of structural remodeling with atrial fibrosis associated with heterogeneity of conduction to the substrate predisposing to AF compared to that due to arrhythmia itself. These dominant components of the AF substrate have since been consistently demonstrated in other models of non-ischemic cardiomyopathy, mitral regurgitation, hypertension, and myocardial infarction. Recent work has also demonstrated that obesity promotes diastolic dysfunction and that together with acutely obstructed respiration, to simulate sleep apnea, promotes LA dilation resulting in AF susceptibility. In addition, prevention of LA distension reduced AF inducibility suggesting an augmenting relationship between the 2 conditions in promoting AF. Acute atrial dilatation or stretch in itself has been demonstrated to result in direction-dependent conduction block in part proposed to reflect an amplification of the anisotropic properties of the atrial myocardium.

Clinical mapping studies have also observed areas of electrical silence, low voltage, fractionated electrograms, and altered and circuitous conduction associated with a predisposition for AF in heart failure, sinus node disease, aging, atrial septal defects, rheumatic heart disease, sleep apnea, and hypertension. Stiles et al.
have demonstrated such findings in patients with lone AF when studied remote from episodes of AF\textsuperscript{225}. Indeed the importance of such atrial structural remodeling has not only been highlighted in the development of arrhythmia but also in determining ablation outcomes\textsuperscript{226} and has recently been implicated in progression of the atrial disease after ablation\textsuperscript{227}.

While population studies have implicated obesity as an important contributor to the increasing epidemic of AF, the current study provides evidence for the progressive evolution of remodeling that has been extensively characterized to form the substrate for AF, with increasing weight gain and obesity. The lack of direct evidence in humans is attributable to the confounding effects of the existence of co-morbid conditions that cluster in obese individuals and are known to contribute to atrial remodeling\textsuperscript{193}. Obese individuals are more likely to suffer concomitant hypertension, DM, obstructive sleep apnea (OSA), coronary artery disease and heart failure – all associated with AF development. The current study observed marked weight-dependent structural and electrophysiological changes translating to a greater AF burden. In the absence of the numerous clinically present comorbidities and the persistence of the abnormalities after controlling for hemodynamic changes, these findings argue in favor of a significant direct contribution of obesity to the AF substrate. In addition, the presence of a greater degree of atrial lipidosis may represent a disease specific component of the structural remodeling predisposing to AF.

**Atrial Tissue Fibrosis**
Tissue fibrosis is the critical structural element leading to abnormal conduction and anisotropy. Previous studies have focused on the central role of TGF-β in promoting fibrosis and atrial fibrillation\textsuperscript{228}. Platelet-derived growth factor has been shown to modulate myofibroblast persistence and promote local tissue microvasculopathy directly and indirectly through TGF-β, via a feedback mechanism. Specifically, PDGF signaling may be particularly important in the pro-fibrotic response of atrial fibroblasts \textsuperscript{158}. Our observation of elevated atrial and systemic pressures with increasing adiposity and the concurrent step-wise elevation in PDGF levels, supports the findings of Iwasaki and Nattel in highlighting the role of atrial stretch in promoting AF in the structurally remodeled atrium\textsuperscript{37}. Importantly, while the above study evaluated the impact of acute stretch in a genetically predisposed obesity model, the current study evaluates the progressive and chronic evolution of obesity due to dietary increase in caloric intake. Whether these models share similar mechanisms to result in atrial remodeling requires further investigation.

Endothelin receptors are expressed in greater abundance on atrial myocardium relative to ventricular myocardium and have been implicated as modulators of the load/stretch-induced hypertrophic response\textsuperscript{178}. Recent studies have demonstrated increased activity of the ET system in overweight and obese humans\textsuperscript{162}. Elevated ET-1 has been shown to predict AF recurrence following an index episode and recurrence following pulmonary vein isolation\textsuperscript{179, 182}. In this study, we have observed increased expression of atrial pro-fibrotic markers, particularly cardiomyocyte ET receptors in the overweight state, persisting at a somewhat lower level with further weight gain.
**Study Limitations**

In this model, independent samples were studied at three distinct points in time. This may introduce confounding factors of intra- and inter-cohort variability. Nevertheless, our model structure was congruent with a previous model of progressive weight gain via an ad-lib feeding regimen\(^{220}\).

Finally, clinical AF is recognized to result from the complex interaction between triggers, perpetuators and substrate\(^{229}\). This study focused on the atrial substrate and did not evaluate other contribution to the development of AF.

**Conclusion**

Progressive obesity predisposes to a greater burden of AF by forming an electro-pathological substrate. This is disproportionate to the progressive hemodynamic impact of obesity and suggests a direct pathogenic role of obesity on the AF substrate.
Acknowledgements

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FIGURE LEGEND

**Figure 1:** Study outline. EPS: electrophysiology study. CMR: cardiac magnetic resonance imaging.

**Figure 2:** Cardiac structural and hemodynamic changes with increasing weight.
Representative CMR cines from each cohort are shown. P values derived from one-way ANOVA. Pair-wise comparisons performed using Bonferroni method. Top panel shows horizontal long-axis and lower panel short-axis. LVEDV left ventricular end-diastolic volume, RVEDV right ventricular end-diastolic volume. MAP mean arterial pressure, LAP left atrial pressure. CMR data presented as mean±SD and hemodynamic data presented as median and IQR.

**Figure 3:** A. Effect of progressive obesity on regional slowing in conduction velocity at the 4 pacing sites. This relationship persisted following adjustment for hemodynamic variables. B. Changes in biatrial conduction heterogeneity index with increasing adiposity. CLs of 500ms and 200ms are presented.
RAA: right atrial appendage, RAFW: right atrial free wall, LAA: left atrial appendage, LAFW: left atrial free wall. CLs of 500ms and 200ms are presented.

**Figure 4:** A. Activation isochronal maps of LAA pacing at 400 msec. Demonstrated are representative examples of S1 (top panel) and S2 (middle panel) for an animal at baseline (left column), overweight (middle column) and obese (right column) cohorts. B. Pacing train (S1) and premature extra stimulus (S2) impact on CV decrement with increasing weight. P values refer to group*condition (S1, S2) interaction.
FIGURE 5: **A.** Scatter plot of spontaneous and induced AF episodes (log transformed) for each animal, and bar graph of AF duration (minutes) with weight cohort (median and IQR; P=0.001). **B.** Example of spontaneous AF observed from the LAA.

**Figure 6:** Histology from atrial tissue (x 200 magnifications). From top panel; Picrosirius red staining, inflammatory infiltrates on H&E and myocardial lipidosis on oil-red-O. Values presented as median and IQR. All pair wise comparisons performed using Mann-Whitney U method.

**Figure 7:** **A.** Western blots of ET$_A$ receptor (63kDa) and ET$_B$ receptor (~30kDa) expression and α-tubulin (55kDa) demonstrates equivalent loading of samples. Two bands are shown per group. **B.** Top panel shows localizing IHC of ET$_A$ receptors and middle panel ET$_B$ receptors, on atrial cardiomyocyte membrane. Lower panel shows IHC staining for ET-1 ligand in cardiomyocyte cytoplasm.

**FIGURE 8:** Immunohistochemical staining for TGF-β1 (top panels), PDGF-BB (middle panels) and CTGF (lowest panels) in atrial tissue and quantitative morphometric.
FIGURE 1: Study Outline
FIGURE 2: Structural changes with weight gain.

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P=0.02

P<0.001
FIGURE 3: Conduction Velocity at 4 sites and 2 CLs.
FIGURE 4: Representative isochronal maps (S1 and S2) for baseline, overweight and obese animals, from the LAA at CL 400msec. and impact of premature beats on CV.
FIGURE 5: Burden of AF with increasing weight and representative spontaneous initiation of AF from left plaque positioned at the LAA

A.

B. Left atrial plaque (LAA)
Figure 6: Atrial Histology
Figure 7: Atrial Endothelin Receptor and Ligand Analysis
Figure 8: Atrial pro-fibrotic mediators
Title of Thesis:

Obesity and Atrial Electrical and Mechanical Remodeling: Implications for Atrial Fibrillation.

Name:

Dr. Hany S. Abed

Chapter 3

Weight and Risk Factor Modification: Impact on Atrial Fibrillation

Short Title: Abed, et al. Weight Management and Atrial Fibrillation

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**Conflict of Interest:** Dr Wittert developed OBEMAN (software) and owns the intellectual property. Kicstart was supplied without charge by Prima Health Solutions. Dr Wittert was paid a consultancy fee during the formulation and development of Kicstart. Prima Health Solutions was a member of the Weight Management Council of Australia of which Dr
Wittert is the Independent Chair. Dr Antic has received lecture fees or research support from Resmed, Phillips Respironics and Fisher and Paykel. Dr Sanders reports having served on the advisory board of Bard Electrophysiology, Biosense-Webster, Medtronic, St. Jude Medical, Merck and Sanofi-Aventis. Dr Sanders reports having received lecture fees or research funding from Bard Electrophysiology, Biosense-Webster, Medtronic and St. Jude Medical.

**Previous Presentation:** Presented in full by Dr Abed and was awarded the American Heart Association Samuel A. Levine Young Clinical Investigator Award 2012, Los Angeles California.
## Statement of Authorship

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**Author Contributions**

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate’s thesis.

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<td>Planning and focused critical evaluation, Study conception</td>
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Date: 17-11-11
ABSTRACT:

Background

Obesity is a risk factor for developing atrial fibrillation (AF). Whether weight and risk factor management can reduce AF burden remains unknown.

Methods

Overweight and obese patients with symptomatic AF were enrolled in a 15-month randomized controlled trial of intensive risk factor management (hypertension, diabetes mellitus, dyslipidemia, smoking, alcohol use and sleep apnea) and either weight reduction (intervention) or general lifestyle advice (control). Primary outcomes were AF burden assessed by the AF Severity Score (AFSS) and 7-day continuous ambulatory rhythm recording. Secondary outcomes were differences in left atrial (LA) size and left ventricular wall thickness measured by echocardiography.

Results

Of 248 patients screened, 150 were enrolled (75/group) and followed for a median of 15 months. There were no baseline differences between groups. Weight decreased from 98.8±13.1 to 80.2±12.5 kg (P<0.001) in the intervention group and from 101±16.4 to 95.9±18.3 kg (P<0.001) in the control group. AF frequency, duration and severity sub-scores decreased more in the intervention than control group, (P< 0.001 for all). On 7-day continuous rhythm recording, there was a decrease in the number of AF episodes (80% vs. 3%, P<0.001) in the intervention as compared to the control group, and a 53% reduction in mean AF duration in the intervention group but a 30% increase in controls (P<0.001). Left ventricular septum thickness (P=0.02) and LA area (P=0.03) decreased in the intervention compared to the control group.
Conclusion

Weight and intensive risk factor management results in a reduction in AF burden and severity with accompanying beneficial cardiac remodeling. (Australia New Zealand Clinical Trials Registration number: ACTRN12610000497000.)
BACKGROUND AND OBJECTIVES:

Atrial fibrillation (AF) has been described as the epidemic of the new millennium, with a projection that by 2050 there will be 12-15 million affected individuals in the USA. Recent data shows an exponential rise in hospitalization for AF. In the USA, the direct economic cost of AF is estimated at $6 billion annually. Although population aging is regarded as an important contributor, obesity and its associated comorbidities may account for a substantial proportion of the increasing prevalence of AF.

Several potential mechanisms exist for the association between obesity and AF, including hypertension, coronary ischemia, diabetes mellitus and obstructive sleep apnea. Recent experimental studies have demonstrated the direct effect of obesity on the atrial structural and electrophysiological substrates predisposing to AF. We therefore evaluated the impact of a structured weight and risk factor management program on AF burden in a randomized controlled trial.

METHODS:

Study Population

Patients were enrolled from referrals to the Centre for Heart Rhythm Disorders of the University of Adelaide. Inclusion criteria were: symptomatic paroxysmal or persistent AF (in, or cardioverted to, sinus rhythm at enrolment); Body mass index (BMI, kg/m²) >27; waist circumference (WC, cm) >100 (male) or >90 (females); and age 21-75 years. Exclusion criteria: serious underlying medical or psychiatric disorder; recent participation (3 months) in a weight loss program; malabsorption; unstable INR (previous hospitalization for reversal of INR or previous anti-thrombin related bleeding); diabetics requiring insulin; valvular...
disease needing intervention; significant endocrine pathology; and inability to provide informed consent. If patients underwent either primary AF ablation or AV node ablation for AF symptom control, then the patient was censored. The study protocol was approved by the Research Ethics Committee of the Royal Adelaide Hospital and the University of Adelaide, Adelaide, Australia. All patients provided written informed consent.

Outcomes
The primary outcome was AF burden, quantified using the AF Severity Score (AFSS; data supplement) and 7 day continuous ambulatory rhythm monitoring. The AFSS is a validated scale that encompasses the domains of AF event frequency, duration and severity\textsuperscript{233}. In addition, it assesses general symptom severity via an associated symptom-specific subscale. The secondary outcomes were LA size and left ventricular wall thickness.

Study Protocol and Design
The study was a single center prospective randomized controlled trial. Eligible subjects were randomized to either a physician-led weight loss group (intervention) or self-directed general lifestyle measures group (control). A software based obesity management system (OBEMAN) developed at the University of Adelaide was used to assess and monitor patients undergoing a tailored weight loss program. Each patient was reviewed 3 monthly.

Blinding
Patients were instructed not to disclose their randomization status. Accordingly, study coordinators and all treating physicians were blinded to subject randomization.

**Weight Management**

**Intervention Group:**

The two phases of the program, weight loss and weight maintenance, followed a previously described approach.\(^{208}\)

*Weight loss phase*

Weight loss was induced over 8 weeks using a modified very low calorie diet (VLCD) (800-1200 Cal/day). Patients were prescribed VLCD meal replacement sachets (Prima Health Solutions, French’s Forest, NSW, Australia) for 2 of their daily meals. The third meal consisted of high animal and plant protein, and low glycemic index (GI), calorie controlled foods. Low intensity exercise was prescribed initially for 20 minutes thrice weekly increasing to 45 minutes thrice weekly.

*Weight maintenance phase*

VLCD meal supplements were gradually phased out and replaced with a low GI meal plan and behavior modification program\(^{206, 234}\) for the following 13 months. Motivational and goal-directed face-to-face visits, 20-40 minutes in duration, were scheduled 3 monthly. Participants could schedule additional visits as required and 24-hour email and telephone contact was available for additional support.

One low intensity activity was replaced by at least 1 moderate intensity activity per week.

Participants in the intervention group were required to maintain a diet, activity, and blood pressure (BP) diary.

**Control Group:**
Written and verbal nutrition and exercise advice was provided at enrollment. Fish oil, 3 grams daily, was prescribed except for participants taking dual antiplatelet agents or vitamin K antagonists because of concerns about additional anticoagulant effects. Completion of a diet and activity diary was not requested. Follow-up was scheduled three monthly.

**Anthropometric Measurements**

Height, using a wall mounted stadiometer, and weight using digital scales, were measured in light clothing without shoes. Body mass index was calculated as weight (kg) divided by the square of the height (meters). Waist circumference was measured at the mid-point between the iliac crest and the lowest rib. Weight and WC were measured 3 monthly.

**Risk Factor Management**

Hypertension, hyperlipidemia, glucose intolerance, sleep apnea, alcohol and tobacco use were screened for and managed in both groups.

If fasting glucose was between 100mg/dL and 125mg/dL, a 2-hour oral glucose tolerance test was performed. Impaired glucose tolerance was managed with lifestyle measures alone, and metformin was added if diabetes was present. Patients with poor glycemic control (HbA1c>7%) were referred to a specific diabetes clinic.

Hyperlipidemia was managed using lifestyle measures, HMG-CoA reductase inhibitors and fibrates (alone or combined) to achieve target values outlined in the National Cholesterol Education Program, Adult Treatment Panel III\(^ {235} \).

Study participants were asked to measure BP 2-3 times daily using home automated monitors and an appropriately sized cuff. For each recording, measurements were
performed 3 times and when 2 systolic values were within 5mmHg, the mean of those readings were entered into the diary. Hypertension was treated using renin-angiotensin-aldosterone system blockers by preference, and other agents where necessary to achieve a BP <130/90mmHg at rest. Doses were titrated to the upper limit prior to adding an additional agent. Twenty four hour ambulatory BP monitoring was performed if home and clinic BP measurements were discrepant over 2 consecutive visits. Changes in the dose and number of anti-hypertensive agents were recorded at each 3 monthly visit.

All patients underwent laboratory-based polysomnography (PSG) with scoring using standard criteria\textsuperscript{236, 237} by qualified registered sleep technicians participating in regular intra- and inter-laboratory external proficiency testing. The decision to treat by a sleep physician was based on symptoms and severity of the disease (generally an apnea hypopnea index >30). Therapies included continuous positive airway pressure (CPAP) or other therapies such as oral appliances or positional changes.

Counseling was provided for smoking cessation and alcohol reduction. The absence of firm guidelines specific for individuals with established serious cardiovascular disease prompted alcohol reduction (≤30g/week) or abstinence as the ultimate goal. Written and verbal counseling was provided with regular follow-up and re-enforcement.

\textit{Metabolic biochemistry}

Fasting blood was drawn at enrollment and follow-up for measurement of serum total cholesterol, HDL, LDL, triglycerides, CRP, insulin and glucose in a NATA certified laboratory using previously described methods\textsuperscript{238}.
Anti-arrhythmic pharmacotherapy

Anti-arrhythmic medications were prescribed for rate and/or rhythm control at the discretion of the treating physician. Electrical cardioversion was performed as a last resort, in preference for pharmacologic cardioversion. Changes in the anti-arrhythmic agents were documented at each 3 monthly visit.

Atrial fibrillation

The AFSS questionnaire was administered at baseline and every 3 months (details in supplement). Continuous ambulatory rhythm recordings were obtained within 7 days of commencing the weight loss phase and repeated at follow-up. They were analyzed, after removal of artifact, by 2 independent observers blinded to participant randomization. Any episode of atrial tachycardia (regular or irregular) of duration ≥30 seconds was included as an arrhythmic episode. Total duration of AF was the cumulative sum of all discrete episodes over the entire recording in minutes.

Cardiac Structure

Two-dimensional transthoracic echocardiography with a 3.5 MHz probe (Vivid7, GE Medical Systems, Horten, Norway) was performed at enrollment and follow-up to measure LA area and ventricular wall thickness. To account for changes in body size without attenuating the effect of obesity LA area was height-indexed. Data were stored digitally and analysis performed offline (EchoPac PC, Version 8 2009, GE Health Care) by an experienced cardiologist, blinded to subject randomization.
Statistics

Power Calculation

Assuming a baseline AF burden score of 13 in both groups, with standard deviations as quoted in the meta-analysis\textsuperscript{240}, and a 35\% subject attrition rate\textsuperscript{241}, 178 subjects would deliver 85\% power to detect a 30\% difference in AF symptom burden score between groups at last follow-up for a 2-sided $\alpha$ of 0.05.

Subject Randomization

Eligible subjects were randomized in a 1:1 ratio to either the control or intervention group using statistical software.

Data Analysis

Differences in outcomes were determined within each group and between the 2 study groups on an intention-to-treat basis. Continuous variables are presented as mean and standard deviation where normally distributed on visual inspection of their histograms, and median (inter-quartile range) otherwise. Categorical variables are summarized as count (proportion). Baseline comparisons were made for continuous parameters using one-way analysis of variance or the Kruskal-Wallis test as appropriate, and by Fisher’s exact test for categorical data. For repeated-measures analysis of continuous dependent variables (which were transformed as required), mixed effects modeling was employed, with subject identity included as a random effect. Patient randomization group (i.e. control vs. treatment) was entered into the mixed model as part of an interaction term with subjects’ visit time. If this group-time interaction term was significant, then it was retained in the model, and it implied that the randomization group influence on the outcome variable was time-dependent. Post hoc testing was then performed to determine whether the group influence
on the dependent variable was significant at each visit. For repeated-measures analysis of binary and highly non-Gaussian dependent variables, an analogous approach was adopted using generalized estimating equations. A binomial probability distribution with logit link function or a Poisson distribution with log link function were assumed as appropriate, as was an autoregressive correlation structure. All statistical tests were 2-sided, and a p-value <0.05 was considered statistically significant. Analyses were undertaken using STATA software, Version 12.1 (Stata Corp, College Station, Texas).

RESULTS

Study participants, baseline characteristics and follow-up

Of 248 consecutive patients with a BMI >27, 178 were eligible and consented. Of these, 28 (10 in the intervention and 18 in the control group) withdrew prior to the program initiation. The final cohort included 150 patients; 75 in the intervention and 75 in the control group [FIGURE1]. Mean follow-up in the control and active groups was 12.0 ± 3.8 and 12.9 ± 2.7 months respectively (median 15 months both groups). At 12 months 73% (109) of subjects had completed the study (52 control and 57 intervention). By 15 months, 54% (81) subjects remained (39 control and 42 intervention). Of the 69 patients not completing 15 months follow-up, 23 underwent catheter ablation; 14 control (6 at 9 months, 2 at 12 months and 6 at 15 months), and 10 intervention (6 at 12 months and 3 by 15 months) patients. One patient in the intervention group underwent catheter ablation of the atrioventricular node followed by permanent pacemaker implantation, for rate control, at 15 months. These were removed from further analysis immediately after undergoing the
procedure. Baseline characteristics are shown in TABLE1, and were similar in the two groups.

**Intervention uptake, pharmacotherapy and risk factor modification**

Fish oil 3g daily was taken as prescribed by 73% of control subjects. From enrollment to study conclusion, there was an increase in use of CPAP (intervention 7% to 28%; controls 9% to 31%), metformin (intervention 1% to 20%; controls 3% to 15%) and lipid lowering agents (intervention 21% to 59%; controls 31% to 59%), P<0.001 for all interventions in both groups. There was a decrease in the proportion of subjects with elevated BP (intervention 85% to 21%; controls 89% to 59%) elevated lipids (intervention 68% to 17%; controls 77% to 40%) and alcohol consumption >30g/week (intervention 33% to 5%; controls 37% to 21%), P<0.001 for all risk factors in both groups. There was a greater decline in hypertension (P=0.02) and excessive alcohol consumption (P=0.01) in the intervention than control group. Number of anti-hypertensive agents increased in the control group (1.4±1.1 at baseline to 1.7±1.0 at 15 months, P=0.02) and decreased in the intervention group (1.3±0.98 at baseline to 1.2±1.2 at 15 months, P=0.03) [FIGURE2] with significant effect of group allocation at 15 months, P=0.005. Serum glucose (P<0.001), insulin (P<0.001), CRP (P≤0.01), total and LDL cholesterol (P<0.001) decreased and HDL (P=0.003) increased, in both groups. Serum triglyceride concentration decreased only in the intervention group (P<0.001). Insulin (P=0.004) and CRP (P<0.001) decreased more in the intervention than control group [TABLE 2].

**Anthropometrics**
Weight, BMI and WC decreased in both groups, but significantly more so in the intervention group [TABLE 2]. This difference was evident by 3 months and persisted for the remainder of follow-up; P<0.001 for group by time interaction for all measures [FIGURE 2].

**Atrial Fibrillation**

**Atrial Fibrillation Severity**

AF episode frequency, duration, severity scores and symptom severity sub-scale all declined in both the control and intervention groups (P≤0.006 all 4 categories) [TABLE 2]. This occurred to a greater extent in the intervention than control group (group-time interaction, P<0.001), evident by 6 months and through to month 15 (P<0.001) [FIGURE 3].

**Continuous Rhythm Monitoring**

Ambulatory rhythm recordings were undertaken at baseline and at final follow up in 109 subjects (52 control, 57 intervention). There were no significant differences between groups at baseline [TABLES 1 and 3]. At final follow up, the proportion having at least one episode (P<0.001), the number of episodes (P<0.001) and cumulative AF duration (P<0.001) were greater in the control than intervention group.

**Cardiac structure**

From baseline to 12-15 months LA area (cm²) decreased 24.1±4.9 to 22.4±5.5 (P=0.001) and 23.1±4.2 to 20.0±3.6 (P<0.001), in the control and intervention groups respectively, with a significant group-time interaction (P=0.03). Height-indexed LA area (cm²m⁻¹) declined from 14.0±2.6 to 12.9±2.9 in the control group (P<0.001) and 13.4±2.4 to 11.5±2.1 in the
intervention group (P<0.001), with a significant group-time interaction (P=0.03). Septal (IVS) thickness (mm) decreased in the control (11.5±2.01 to 10.9±1.80, P<0.001), but more in the intervention (11.2±1.67 to 10.1±1.57, P<0.001) group (group-time interaction P=0.02). Posterior wall thickness (mm) also decreased in the control (10.2±1.52 to 9.77±1.34 [P<0.001]), but more in the intervention (10.1±1.28 to 9.19±1.16 [P<0.001]) group (group-time interaction P=0.007).

**Safety**

Infrequent adverse events were seen in both groups as shown in TABLE4. Common adverse events were INR instability. One patient was withdrawn for a persistently sub-therapeutic INR <2.0 and one for supra-therapeutic INR level. No serious bleeding was observed in either group. Postural symptoms, which frequently occurred in patients with SBP<100mmHg or a postural drop >10mmHg, resolved with reduction in antihypertensive medications in all cases.

**DISCUSSION**

This study shows that a structured weight management program for highly symptomatic patients with AF reduced the burden and severity of AF when compared with attempts at optimally managing risk factors alone. In conjunction there was a reduction in the use of anti-arrhythmic agents. Previous epidemiological data has suggested a 4-5% increased risk of developing AF with each unit increment in BMI. In our cohort, the intervention group had a mean weight reduction of 10kg, or 3.5 kgm⁻² BMI units, greater than in the control
group. This was accompanied by less alcohol consumption and hypertension as compared to the control group.

Obesity, hypertension, impaired glucose tolerance/diabetes mellitus, and OSA are closely inter-related manifestations of the metabolic syndrome and all have been previously identified as independent risk markers for AF. As observed in the current and prior studies, an active intervention directed primarily at weight loss has significant favorable impact on other components of this syndrome.

Hypertension has been estimated to increase the risk of developing AF by 70-80%\textsuperscript{242}. The effect of hypertension management on AF outcomes has been evaluated in multiple studies and in different patient groups. Most, but not all\textsuperscript{54}, studies have suggested a modest preventative effect on AF when renin-angiotensin active agents\textsuperscript{25, 243, 244} are used in the presence of structural heart disease\textsuperscript{56}, including left ventricular hypertrophy, or for the maintenance of sinus rhythm following cardioversion\textsuperscript{245}. These studies however have not examined the concurrent management of comorbidities often seen in hypertensive patients, such as sleep apnea and alcohol consumption\textsuperscript{246}. Furthermore, the influence of alcohol consumption on AF risk was estimated by Kodama and colleagues as 8% per 10g daily consumption increment, without a safe consumption threshold.\textsuperscript{74} Sparse mechanistic studies have suggested atrial conduction abnormalities with low dose alcohol\textsuperscript{77}, thus warranting its consideration as an independent AF associated risk factor. Therefore individually and in association, both hypertension and alcohol consumption may represent modifiable risk factors imparting a substantial risk of AF.
Although epidemiological data has found no significant association between insulin resistance and AF\textsuperscript{247}, diabetes mellitus has been shown to increase the risk of new-onset AF by up to 50\%\textsuperscript{248}. The basis of this may be the influence of plasma insulin and an obesogenic diet on myocardial energetics and function\textsuperscript{249,250}. Additionally, an independent association between CRP and AF has been established\textsuperscript{251}, with a 36\% increased risk with each CRP level tertile increment\textsuperscript{252}. In our cohort, we observed a 60\% and 48\% reduction in serum insulin and CRP, respectively, in the intervention group, as might be expected in response to the weight loss.

Obesity, hypertension and OSA have been independently associated with atrial dilatation which has been demonstrated to influence AF initiation, maintenance and progression, with an augmenting effect on the obesity-attributable risk\textsuperscript{18,19,224}. Atrial dilatation can be reversed to a variable extent with aggressive management of risk factors\textsuperscript{81,253}. Importantly, a reduction in LA size through hypertension management has been shown to have a favorable effect on new-onset AF risk\textsuperscript{254}. It is likely that the improvement in each of the risk factors, particularly hypertension, contributed to the observed reduction in LA size, similar to observations by Gottdiener and colleagues\textsuperscript{255}.

Atrial fibrillation is an arrhythmia with a significant impact on quality of life and survival\textsuperscript{256}. Our results suggest that weight reduction and improvement of multiple cardio-metabolic risk factors, translated into dynamic changes in cardiac structure and subsequently AF burden. The lifestyle and comprehensive metabolic risk factor management program was feasible to deliver, effective, associated with a limited risk of serious adverse events, and resulted in a substantial reduction in the burden and symptom severity of AF. In addition,
this approach provides scope for AF disease regression by addressing individual components promoting the proarrhythmic substrates.

**Study Limitations**

Our study has several limitations. This single center study was undertaken in primarily a Caucasian male (67%) population and may have limited applicability to non-white ethnic subgroups or females. There were a number of dropouts, but they were similar between groups. Methods to optimize program delivery and participant retention are required.

Although a combination of 7-day continuous ambulatory recording and a validated questionnaire may have wider clinical applicability in an ambulatory setting, an implanted monitoring device may have provided a more accurate assessment of arrhythmia burden.

**Conclusion**

A comprehensive risk factor management program with emphasis on weight reduction resulted in a significant reduction in AF burden and symptom severity in conjunction with favorable changes in cardiac remodeling and serum cardio-metabolic markers. Whether this approach, and the extent to which it can be maintained, alters the natural history of AF in the longer term remains to be determined.
Acknowledgements

Dr Paul Dorian of the University of Toronto, Department of Medicine, for provision of the Atrial Fibrillation Severity Score (AFSS) questionnaire utilized in this study.

Prima Health Solutions (Frenchs Forest, NSW, Australia) for providing Kickstart VLCD meal replacement products and equipment.
### TABLE 1: Baseline characteristics of both study groups

<table>
<thead>
<tr>
<th></th>
<th>Control Group (N=75)</th>
<th>Intervention Group (N=75)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>62 (54-68)</td>
<td>62 (55-65)</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Male gender, n (%)</strong></td>
<td>50 (67)</td>
<td>51 (68)</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Anthropometric Measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>112±10.9</td>
<td>110±9.51</td>
<td>0.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>101±16.4</td>
<td>98.8±13.1</td>
<td>0.3</td>
</tr>
<tr>
<td>BMI (Kgm⁻²)</td>
<td>33.8±4.07</td>
<td>32.8±3.54</td>
<td>0.1</td>
</tr>
<tr>
<td>BSA (m²) [Mosteller estimation method]</td>
<td>2.2±0.23</td>
<td>2.2±0.18</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Metabolic Risk Factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excess alcohol consumption (&gt;30g/week), n (%)</td>
<td>28 (37)</td>
<td>25 (33)</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Smoker, n (%)</strong></td>
<td></td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>No</td>
<td>45 (60)</td>
<td>43 (57)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>5 (7)</td>
<td>2 (3)</td>
<td></td>
</tr>
<tr>
<td>Reformed</td>
<td>25 (33)</td>
<td>30 (40)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>65 (87)</td>
<td>62 (83)</td>
<td>0.7</td>
</tr>
<tr>
<td>Diabetes mellitus/Impaired glucose tolerance</td>
<td>21 (28)</td>
<td>18 (24)</td>
<td>0.7</td>
</tr>
<tr>
<td>Hyperlipidemia, n (%)</td>
<td>51 (68)</td>
<td>45 (60)</td>
<td>0.4</td>
</tr>
<tr>
<td>Coronary artery disease, n (%)</td>
<td>10 (13)</td>
<td>7 (9)</td>
<td>0.6</td>
</tr>
<tr>
<td>Valvular heart disease, n (%)</td>
<td>4 (5)</td>
<td>5 (7)</td>
<td>0.9</td>
</tr>
<tr>
<td>Obstructive sleep apnea (OSA), n (%)</td>
<td>52 (84)</td>
<td>55 (89)</td>
<td>0.6</td>
</tr>
<tr>
<td>OSA moderate-severe, n (%)</td>
<td>32 (52)</td>
<td>30 (48)</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>AHI</strong></td>
<td>23.5±15.4</td>
<td>22.8±13.2</td>
<td>0.8</td>
</tr>
<tr>
<td><strong>Medication Use</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of anti-arrhythmic agents, n (%)</td>
<td></td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>0</td>
<td>2 (3)</td>
<td>6 (8)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>42 (56)</td>
<td>40 (53)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>31 (41)</td>
<td>29 (39)</td>
<td></td>
</tr>
<tr>
<td>Number of anti-hypertensive agents, n (%)</td>
<td></td>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td>0</td>
<td>17 (23)</td>
<td>12 (16)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>27 (36)</td>
<td>37 (49)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>17 (23)</td>
<td>17 (23)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10 (13)</td>
<td>6 (8)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4 (5)</td>
<td>3 (4)</td>
<td></td>
</tr>
<tr>
<td><strong>Echocardiographic Measures</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA area (cm²)</td>
<td>24.2±4.8</td>
<td>23.2±4.1</td>
<td>0.2</td>
</tr>
<tr>
<td>LA area indexed (cm²/m⁻²)</td>
<td>14.0±2.6</td>
<td>13.4±2.4</td>
<td>0.1</td>
</tr>
<tr>
<td>LV septum (mm)</td>
<td>11.0±2.2</td>
<td>11.0±1.7</td>
<td>0.3</td>
</tr>
<tr>
<td>LV posterior wall (mm)</td>
<td>10.0±1.5</td>
<td>10.0±1.3</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Atrial Fibrillation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paroxysmal AF, n (%)</td>
<td>42 (56)</td>
<td>44 (59)</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Atrial Fibrillation Severity Scale (AFSS)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency [1-10]</td>
<td>7.3±1.9</td>
<td>7.1±1.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Duration [1-10]</td>
<td>7.4±2.4</td>
<td>7.1±2.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Severity [1-10]</td>
<td>6.9±2.0</td>
<td>6.8±2.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Symptom [0-35]</td>
<td>16±7.4</td>
<td>15±6.9</td>
<td>0.5</td>
</tr>
<tr>
<td>Duration of AF (months)</td>
<td>60 (36-120)</td>
<td>60 (36-118)</td>
<td>0.4</td>
</tr>
<tr>
<td>Longest Episode (hours)</td>
<td>48 (10-49)</td>
<td>26 (8-72)</td>
<td>0.9</td>
</tr>
<tr>
<td>Continuous ambulatory recording duration (hrs)</td>
<td>75.9 (31-112.7)</td>
<td>85.9 (24.0-112.3)</td>
<td>0.4</td>
</tr>
<tr>
<td>AF episode number (≥30sec.)*</td>
<td>1 (0-4)</td>
<td>1 (0-2)</td>
<td>0.8</td>
</tr>
<tr>
<td>AF duration cumulative (mins)</td>
<td>381 (0-1440)</td>
<td>253 (0-1462)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Table 1:*Number of AF/atrial arrhythmia episodes recorded over a 7 day-continuous ambulatory rhythm recording. An episode is defined as an AF/atrial arrhythmia episode of duration ≥30seconds.
TABLE 2: Anthropometric, AF severity and biochemical changes.

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Intervention Group</th>
<th>Control Group</th>
<th>Intervention Group</th>
<th>Control Group</th>
<th>Intervention Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (N=75)</td>
<td>Follow-up‡ (N=39)</td>
<td>P value*</td>
<td>Baseline (N=75)</td>
<td>Follow-up‡ (N=42)</td>
<td>P value*</td>
</tr>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>112±10.9</td>
<td>107±12.8</td>
<td>&lt;0.001</td>
<td>110±9.51</td>
<td>92.8±10.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>101±16.4</td>
<td>95.9±18.3</td>
<td>&lt;0.001</td>
<td>98.4±13.1</td>
<td>80.2±12.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (Kgm⁻²)</td>
<td>33.8±4.07</td>
<td>32.5±4.62</td>
<td>&lt;0.001</td>
<td>32.8±3.54</td>
<td>27.2±2.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Atrial Fibrillation Severity Score (AFSS)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AF frequency [1-10]</td>
<td>7.3±1.9</td>
<td>6.6±2.1</td>
<td>0.002</td>
<td>7.1±1.6</td>
<td>3.3±1.2</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>AF duration [1.25-10]</td>
<td>7.4±2.4</td>
<td>6.3±2.8</td>
<td>0.002</td>
<td>7.1±2.0</td>
<td>2.4±1.8</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>AF episode severity [1-10]</td>
<td>6.9±2.1</td>
<td>5.8±2.2</td>
<td>0.001</td>
<td>6.8±2.2</td>
<td>3.1±1.7</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>AF symptom subscale [0-35]</td>
<td>16±7.4</td>
<td>14±7.2</td>
<td>0.006</td>
<td>15±6.9</td>
<td>6.7±5.4</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td><strong>Serum Biochemistry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>104.4±21.6</td>
<td>100.8±18.0</td>
<td>&lt;0.001</td>
<td>104.4±25.2</td>
<td>93.6±25.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>33.7±10.8</td>
<td>21.2±10.6</td>
<td>&lt;0.001</td>
<td>33.9±14.2</td>
<td>13.5±6.60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>28.8±12.6</td>
<td>28.8±14.4</td>
<td>0.2</td>
<td>27.0±12.6</td>
<td>23.4±10.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>86.4±21.6</td>
<td>81.0±19.8</td>
<td>&lt;0.001</td>
<td>82.8±19.8</td>
<td>79.2±16.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>21.4±6.1</td>
<td>22.5±6.8</td>
<td>0.003</td>
<td>21.1±6.3</td>
<td>23.4±6.1</td>
<td>0.003</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>54.0±19.8</td>
<td>48.6±18.0</td>
<td>&lt;0.001</td>
<td>50.4±18.0</td>
<td>45.0±14.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>2.3±1.5</td>
<td>1.9±1.4</td>
<td>0.01</td>
<td>2.5±1.6</td>
<td>1.3±0.93</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2: Changes in anthropometric measures, AF burden scores and serum biochemistry from baseline to follow-up. *P value refers to within group difference (baseline to follow-up), †P value refers to between group difference over time (group-time interaction). ‡Median follow-up 15 months for both groups.
TABLE 3: Atrial fibrillation detected by 7-day continuous ambulatory rhythm recording.

<table>
<thead>
<tr>
<th></th>
<th>Proportion having ≥ one episode of AF*</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (N=150)</td>
<td>Follow-up (N=109)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>58%</td>
<td>57%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td>66%</td>
<td>22%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Number of AF episodes*</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (N=150)</td>
<td>Follow-up (N=109)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.7±0.66</td>
<td>3.6±0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td>4.5±0.98</td>
<td>0.93±0.28</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Total AF Duration (minutes)*</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (N=150)</td>
<td>Follow-up (N=109)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1765±369</td>
<td>2289±546</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td>1534±270</td>
<td>731±252</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Data represented as mean ± standard error. *Derived from 7-day continuous rhythm recordings. †P values represent differences between the 2 study groups as reflected by the group-time interaction. Median follow-up 15 months both groups.
<table>
<thead>
<tr>
<th>Stage</th>
<th>Event</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 months</td>
<td>1 severe anemia (Hemoglobin &lt;90 g/dL)</td>
<td>Due to diabetic renal disease</td>
</tr>
<tr>
<td></td>
<td>1 acute coronary syndrome</td>
<td>Managed invasively</td>
</tr>
<tr>
<td></td>
<td>1 progressive acute</td>
<td>Secondary to atrial tachyarrhythmia cardiomypathy. Underwent AV node ablation and permanent pacemaker insertion.</td>
</tr>
<tr>
<td></td>
<td>decompensated cardiac failure</td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>1 thyrotoxicosis</td>
<td>All de-novo biochemical and clinical. Assumed due to amiodarone. Required prolonged corticosteroids and carbimazole</td>
</tr>
<tr>
<td></td>
<td>2 thyrotoxicosis</td>
<td>Thought to be recurrence of ovarian cancer (&gt;5 years prior)</td>
</tr>
<tr>
<td></td>
<td>1 malignancy</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 gastrointestinal symptoms</td>
<td>1 bloating, 1 diarrhea and 1 constipation. All failed conservative management</td>
</tr>
<tr>
<td></td>
<td>2 unstable INR</td>
<td>1 elevated INR (&gt;9) required IV vitamin K, 1 unable to achieve therapeutic INR due to dietary interference</td>
</tr>
<tr>
<td>15 months</td>
<td>1 acute coronary syndrome</td>
<td>Managed invasively</td>
</tr>
<tr>
<td></td>
<td>1 severe depression</td>
<td>Known pre-existing stable treated depression</td>
</tr>
<tr>
<td></td>
<td>1 severe refractory</td>
<td>Pre-existing hypertension. Persistent readings &gt;190/110 despite multiple agents. Referred for investigation of secondary causes</td>
</tr>
<tr>
<td></td>
<td>hypertension</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 malignancy</td>
<td>Renal cell</td>
</tr>
<tr>
<td></td>
<td>1 troponin negative chest</td>
<td>Stable coronary artery disease. Cardio-respiratory causes excluded</td>
</tr>
<tr>
<td></td>
<td>pain</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 progressive acute</td>
<td>Progression of chronic ischemic coronary artery disease. Managed medically.</td>
</tr>
<tr>
<td></td>
<td>decompensated cardiac failure</td>
<td></td>
</tr>
</tbody>
</table>
Figure Legend

**FIGURE1**: Patient recruitment, attrition and retention flow-chart.

**FIGURE2**: Changes in body weight (kg), body mass index (kgm$^{-2}$), waist circumference and differences in the number anti-arrhythmic (AA) agents and anti-hypertensive (AHT) agents between the study groups over the follow-up duration. * P<0.05, ** P<0.01, *** P<0.001.

**FIGURE3**: Changes in Atrial Fibrillation Severity Score (AFSS) components between the 2 study groups, over the study follow-up duration. * P<0.05, ** P<0.01, *** P<0.001.
FIGURE 1: Patient flow and retention outline

248 subjects assessed for eligibility
178 eligible subjects consented after explanation of study outline
150 Randomized consecutively
28 Declined to participate

75 Randomized to Control group

75 Control
72 Controls
3 Voluntary withdrawal from study

61 Controls
6 AF ablation, 2 voluntary withdrawal, 1 progressive cardiomyopathy, 1 severe anemia, 1 acute coronary syndrome

52 Controls
4 voluntary withdrawal, 2 AF ablation, 1 gastritis, 1 thyrotoxicosis, 1 re-located interstate

39 Controls
2 lap-band surgery, 1 acute coronary syndrome, 3 voluntary withdrawal, 1 severe depression, 1 severe hypertension, 6 AF ablation

75 Randomized to Intervention group

75 Intervention
3 months
6 months
9 months
12 months
15 months

73 Intervention
2 non-compliance with study protocol

57 Intervention
6 AF ablation, 2 unstable INR, 3 gastro-intestinal symptoms, 1 voluntary withdrawal, 1 lap-band surgery, 2 thyrotoxicosis, 1 diagnosed with malignancy

42 Intervention
3 AF ablation, 1 AV node ablation and pacemaker implant, 6 voluntary withdrawal, 1 diagnosed with malignancy, 1 re-located interstate, 1 persistent chest pain (unknown cause), 1 progressive cardiomyopathy, 2 enrolled in a commercial weight loss program, 1 non-compliance with study protocol

70 Excluded
(26 Declined participation)
(44 Exclusion criteria met)
FIGURE 2: Changes in Anthropometric measurements, anti-arrhythmic (AA) and anti-hypertensive (AHT) agent usage throughout study period, in both groups over the study duration.
FIGURE 3: Changes in self-assessed AF burden using the Atrial Fibrillation Severity Score (AFSS) algorithm
Supplement

AFSS Questionnaire

NOTE:
This supplement is included on page 137 of the print copy of the thesis held in the University of Adelaide Library.
Title of Thesis:

Obesity and Atrial Electrical and Mechanical Remodeling: Implications for Atrial Fibrillation.

Name:

Dr. Hany S. Abed

Chapter 4

Impact of Weight Reduction on Pericardial Fat and Cardiac Structure in Patients with Atrial Fibrillation

Short Title: Weight loss and pericardial fat

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Stephen G. Worthley, MBBS, PhD*; Darryl P. Leong, MBBS, MPH, PhD*†;

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Conflict of Interest: Kicstart was supplied without charge by Prima Health Solutions. Dr Wittert was paid a consultancy fee during the formulation and development of Kicstart. Prima Health Solutions is a member of the Weight Management Council of Australia of which Dr Wittert is the Independent Chair.

Presentation: Accepted for presentation at the American College of Cardiology (ACC) Annual Scientific Sessions Young Investigator Award competition. To be presented by Dr Abed at the American College of Cardiology Young Investigator Award 2013, San Francisco California.


### Statement of Authorship

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**Author Contributions**

By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate's thesis.

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<td>Stephen J. Warfuly</td>
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Abstract

Objectives
We sought to determine the effect of weight reduction on pericardial fat volume (PF), atrial fibrillation (AF) severity and cardiac structure, in overweight and obese patients.

Background
Pericardial fat and obesity have been independently associated with AF. Both factors have been shown to predict atrial enlargement and worse disease outcomes.

Methods
We prospectively randomized 103 patients into either a structured weight management program, or a control group receiving lifestyle counseling. Both groups underwent Atrial Fibrillation Severity Score (AFSS) assessment. We determined the change in AFSS, PF, atrial volumes and myocardial mass (MM) using cardiac MRI at baseline and 12 months follow-up.

Results
At 12 months 69 patients completed the study. Weight loss was greater in the intervention group (13.6±5.4kg) compared to controls (5.8±8.7kg), P<0.001. Left atrial (LA) and right atrial (RA) maximal volumes decreased in the intervention group (LA: 8.7±8.8mL, RA: 12.2±9.4mL, decline) compared to a small increase in the control group (LA: 0.2±2.6mL, 0.1±3.6mL), P<0.001 for both atria. There was a significant decline in PF and MM in the intervention group (MM: 14.5±7.0g, PF: 22.0±15.8cm³), compared to an increase in controls (MM: 2.4±6.7g, PF: 4.0±6.9cm³), P<0.001, however no change in ventricular volumes or function was observed. There was a significant decline in the intervention group AFSS (P<0.001), compared to controls. Of all adiposity measures, PF was the strongest predictor of a favorable improvement in AFSS (P=0.009).
Conclusion

Weight reduction is associated with favorable changes in PF volume, atrial size and myocardial mass. Pericardial fat volume is a significant predictor of AF severity.

(Trial registration: ACTRN12610000497000.)
Introduction

Obesity is recognized as a significant risk factor for the development and progression of AF\textsuperscript{7,14}. Whilst conventional obesity-associated metabolic conditions have been mechanistically implicated, novel risk factors have recently emerged as potential intermediaries\textsuperscript{92,195,257}. Pericardial fat has been shown to predict the development of AF independent of traditional risk factors including measures of systemic obesity, such as body mass index (BMI)\textsuperscript{93,94}. Epidemiological observations suggest PF may be a metabolically active depot, strongly correlated with markers of systemic inflammation, in addition to influencing contiguous cardiac pathological changes\textsuperscript{258} in view of the direct contact between the epicardial fat layer and underlying myocardium\textsuperscript{259}. Further, left atrial (LA) size has been shown to be a marker for the development of AF and its progression, in a relationship that is further compounded by the presence of obesity\textsuperscript{18}. The aim of this current study was therefore to determine whether PF stores and atrial volumes are favorably responsive to weight loss, in a prospective interventional weight reduction program, and the impact of this depot on indices of AF severity.

Methods

Study design and patient population

The obesity and AF study is a multi-component study into the relationship between obesity and AF. Patients were enrolled and consecutively randomized from Cardiovascular Center, the outpatient service of the Center for Heart Rhythm Disorders, The University of Adelaide. Cardiac MRI scans were performed at the Cardiovascular Investigation Unit of The Royal Adelaide Hospital. The MRI component of the obesity and AF study is presented here.
Patients with a BMI (kgm\(^{-2}\))>27; waist circumference (WC, cm) >100 (male) or >90 (females); and age 21-75 years with symptomatic paroxysmal or persistent AF (in, or cardioverted to, sinus rhythm at enrolment) were eligible for study recruitment. Exclusion criteria were: serious underlying medical or psychiatric disorder; recent participation (3 months) in a structured weight loss program; malabsorption disorder; unstable INR (previous hospitalization for reversal of INR or previous anti-thrombin related bleeding); left ventricular ejection fraction ≤35%; insulin-dependent diabetes mellitus; valve disease needing intervention; significant endocrine pathology; and inability to provide informed consent.

**Intervention group**

The intervention group was enrolled in an 8 week very low calorie diet (VLCD) utilizing meal replacement sachets (Kickstart, Prima Health Solutions) for 2 of 3 daily meals. The third meal consisted of a low glycemic index (GI), calorie controlled meal. Following the 8 week VLCD period, the meal replacement sachets were phased out and focus was placed on maintained weight loss through permanent lifestyle change. Physical activity was prescribed at weekly, 3x 20 minutes moderate intensity activities gradually increased to 3x 45 minutes. Intensive dietary and exercise counseling was provided through minimum 3-monthly physician contact with additional 24 hour telephone and email support. Patients were instructed to maintain a detailed lifestyle journal documenting nutritional intake (content, amount and preparation) and exercise (intensity, duration and type), for review at each clinical encounter.

**Control group**
Control patients were issued with once-off written nutrition educational material and 3-monthly follow-up counseling. Exercise and nutrition was largely self-directed. Additionally, patients were prescribed 3g marine triglyceride capsules daily.

Weight and waist circumference measurement

Weight, height, BMI and WC were recorded using digital scales and a stadiometer, in accordance with the National Health and Nutrition Examination Survey (NHNES III) anthropometrics recommendations. Body surface area (BSA) was calculated using the mosteller equation.

Atrial Fibrillation Severity

The severity of AF was assessed at baseline and 12 months, using the validated semi-quantitative questionnaire, University of Toronto AFSS\textsuperscript{260}. This provided an assessment in the domains of AF frequency, duration and severity, each standardized to a score out of 10, in addition to a symptom-specific questionnaire scored out of 35.

Blood pressure (BP) measurement

Patients were instructed to measure non-invasive BP morning and evening for 10 days using an automated monitor. The mean systolic and diastolic blood pressures (SBP and DBP) over these 10 days were calculated, prior to clinical review, to guide anti-hypertensive medication management and titration.

Insulin resistance and inflammatory markers
Fasting plasma glucose, insulin and C-reactive protein levels were measured at baseline and repeated at 12 months follow-up. The homeostatic model assessment was used to calculate the index of insulin resistance (HOMA-IR).

Cardiac MRI protocol and analysis

**CMR acquisition**

Cardiovascular magnetic resonance imaging was performed on subjects using a 1.5T clinical magnetic resonance scanner (Siemens Avanto, Erlangen, Germany) and a standard phased array surface coil with subjects in the supine position.

For the ventricular image set, long-axis reference views were used for positioning the 8 to 12 ventricular short-axis slices from the level of the mitral valve to LV apex. Images were obtained during breath-hold (8 to 10 seconds) with retrospectively ECG-gated SSFP (Steady-State Free Precession) sequences: image matrix 256 X 150, field of view (FOV) 380mm, repetition time 52.05ms, echo time 1.74ms, flip angle 70°. Standard ventricular short axis slices were 6 mm thick with 4mm intersection gaps. For the atrial image set, contiguous slices in both short (bi-atrial) and horizontal long axis views (four chamber) were obtained with slice thickness of 6 mm and no intersection gap.

**Ventricular analysis**

Ventricular analysis was performed off-line using proprietary software program (Argus software, Siemens, Germany). Left and right ventricular (LV and RV) chambers were manually traced at end-diastole (start of R-wave) and in end-systole (smallest cavity area). Left ventricular basal slice selection was determined by at least 50% of ventricular
myocardium surrounding the blood pool\textsuperscript{263}. This technique has previously been shown to be highly accurate and reproducible\textsuperscript{264} with intra-class coefficients for intra- and inter-observer variability from our own group of 0.7 and 0.77 for RV measurements and 0.91 and 0.94 for LV measurements\textsuperscript{265}. With regard to right ventricular basal slices, as per previously published methods, only the volume below the level of the pulmonary valve was included if the valve was seen\textsuperscript{266}.

\textit{Atrial analysis}

Atrial chambers were manually traced utilizing a disc summation method described previously at ventricular end-systole and end-diastole\textsuperscript{265}. The borders of the LA were defined as the plane of the mitral valve and the visually apparent juncture of LA with pulmonary veins. The borders of the RA were defined as the plane of the tricuspid valve and the juncture with the caval veins. Intra-class correlation coefficients for intra- and inter-observer variability were documented at 0.98 and 0.92 for RA and 0.87 and 0.84 for LA volumes respectively\textsuperscript{265}.

\textit{Pericardial adipose tissue quantification}

Pericardial adipose tissue was traced at end-diastole from the ventricular short-axis stack as described previously\textsuperscript{267}. In summary, traces were made around the myo-epicardial border and outermost border of adipose tissue, subtending an area of PF. Traces were undertaken on contiguous ventricular slices and PF volume subsequently derived using a disc summation method. Strong intra- and inter-observer agreement was shown, with correlation coefficients of 3.5 and 4.9\% respectively\textsuperscript{267}.

\textbf{Statistical analysis}
Statistical tests were 2-sided, and a P-value <0.05 was considered statistically significant. Differences in outcomes were determined within each group and between the 2 study groups. Continuous variables are shown as mean and standard deviation, and median (interquartile range) otherwise. Categorical variables are summarized as proportions. Baseline comparisons were made for continuous parameters using one-way analysis of variance or the Kruskal-Wallis test as appropriate, and by Fisher’s exact test for categorical data. For repeated-measures analysis of continuous dependent variables, a linear mixed-effects model was employed, with subject identity included as a random effect. Patient randomization “group” (i.e. control vs. treatment) was entered into the mixed model as part of an interaction term with subjects’ visit time. If this group-time interaction term was significant, then it was retained in the model, and it implied that the randomization group influence on the outcome variable was time-dependent. For post-hoc analysis, Pearson’s correlation coefficient ($r$) was used to explore relationships between the adiposity, structural and metabolic variables. To identify independent predictors of the reduction in AF burden following weight loss, difference in AFSS scores (frequency, duration and severity) from baseline to last follow-up were entered into a linear regression model as outcome variables against the differences in the following predictor variables (also from baseline to last follow-up); metabolic measures (SBP, DBP, HOMA-IR, C-reactive protein), adiposity measures (BMI, BSA, PF, WC) and structural measures (MM, maximal LA volume). Multilinear regression was then undertaken to identify the strongest predictors of the reduction in AF burden following weight loss. Candidate univariate predictor variables were those with a univariate P-value ≤0.2, and regression was undertaken using backward elimination. All
analyses were performed using STATA software, Version 12.1 (Stata Corp, College Station, Texas).

Results
At 12 months follow-up, 69 patients had completed the study follow-up and had undergone a follow-up scan. As shown in FIGURE1, 1 patient declined a second scan, 10 voluntarily withdrew participation citing claustrophobia, 16 underwent a cardiac device implant and 7 were excluded due to poor study images rendering them unable to be analyzed in detail for the study purposes. Baseline characteristics are shown in TABLE1.

Weight reduction
FIGURE2 shows the changes in anthropometric measures. In the control group, weight decreased from 102.6±15.4kg at baseline to 98.7±16.3kg at 12 months (P=0.01) and BMI from 33.9±3.7 kgm$^{-2}$ to 32.4±4.7 kgm$^{-2}$ (P=0.01). The intervention group achieved a weight reduction from 101.5±12.7kg to 86.5±13.5kg (P<0.001) and BMI from 32.6±3.5 kgm$^{-2}$ to 28.0±3.5 kgm$^{-2}$ (P<0.001), over the study duration.

Waist circumference and BSA decreased from 111.4±11.1cm to 106.9±12.8cm (P=0.002) and 2.20±0.22m$^2$ to 2.18±0.22m$^2$ (P=0.6) in the control group, respectively. In the intervention group, WC and BSA decreased from 111.0±9.8cm to 96.2±11.5cm (P<0.001) and 2.23±0.17m$^2$ to 2.06±0.19m$^2$ (P=0.07), respectively. The differences between the groups were significant over time for weight (group x time interaction P=0.01), BMI (group x time interaction P=0.001) and WC (group x time interaction P=0.004).
Insulin resistance, blood pressure and C-reactive

The metabolic changes are shown in TABLE2. The HOMA–IR (units) decreased from 8.4±3.4 to 4.6±2.8 in the control group (P<0.001) and 9.7±4.3 to 3.8±2.5 (P<0.001) in the intervention group. The group x time differences trended towards significance (P=0.07). C-reactive protein (mg/L) decreased from 2.3±1.4 to 1.7±1.1 (P=0.1) in the control group and from 2.3±1.8 to 1.3±1.0 (P<0.001) in the intervention group, with a group x time difference also trending towards significance (P=0.08).

Mean SBP declined from 134±18mmHg to 133±17mmHg (P=0.3) and DBP from 82±12mmHg to 81±11mmHg (P=0.4), in the control group. The intervention group showed a decline in SBP from 134±14mmHg to 130±13mmHg (P<0.001) and DBP from 81±8 to 79±8mmHg (P<0.001). The mean SBP showed a significant group x time difference (P=0.001), but not the mean DBP (P=0.2).

AF severity scores

TABLE2 shows the changes in AF burden (frequency, duration, severity) and AF symptom severity scale. The control group showed a small reduction across all AFSS parameters; however this did not reach significance. The intervention group, showed a significant decline in AFSS, with a significant group x time interaction (P<0.001).

Pericardial fat

Structural changes are shown in TABLE3. Subjects in the control group showed a marginal but significant increase in PF volume from 143.2±52.3cm³ to 147.2±51.5cm³ (P=0.002), while the intervention group had a significant reduction in the pericardial fat volume.
140.9±34.2cm³ to 118.8±31.8cm³ (P<0.001). The group x time significance at 12 months was P=0.01.

**Atrial volumes**

Over the study duration, the intervention group demonstrated a reduction in atrial volumes, whereas the control group showed minimal change. At baseline, the control group maximal LA and RA volumes were 108.8±25.7cm³ and 98.0±19.9cm³, respectively, and at 12 months the maximal LA and RA volumes were 108.9±25.6cm³ (P=0.7) and 98.1±20.6cm³ (P=0.9), respectively. The intervention group showed a reduction of LA volume from 105.0±18.0cm³ to 96.4±14.1cm³ (P<0.001) and RA volume from 98.7±19.2cm³ to 86.5±15.8cm³ (P<0.001). The group x time interaction terms were significant; LA P=0.01 and RA P=0.01.

To account for the influence of body size on atrial size, without attenuating the effect of obesity, we indexed maximal atrial volume to height. The control group showed a change in indexed LA volume from 62.4±13.6cm³m⁻¹ to 62.5±13.5cm³m⁻¹ (P=0.7) and indexed RA volume from 56.3±10.7cm³m⁻¹ to 56.4±10.9cm³m⁻¹ (P=0.9). The intervention group had a reduction in indexed LA volume from 59.6±10.1cm³m⁻¹ to 54.7±8.2cm³m⁻¹ (P<0.001) and indexed RA volume from 55.8±9.7cm³m⁻¹ to 49.0±8.2cm³m⁻¹ (P<0.001), with significant group x time differences; LA P=0.005 and RA P=0.002. The same trends were seen for minimal atrial volumes indexed for height; LA P=0.007, RA P = 0.003.

**Ventricular volumes**

Left ventricular end diastolic volume (LVEDV) decreased from 172.0±50.4cm³ to 170.9±48.9cm³ (P=0.7) and RVEDV increased from 144.2±39.3cm³ to 146.7±39.3cm³ (P=0.4), in the control group. The intervention group LVEDV decreased from 167.2±41.9cm³ to 163.9cm³ (P=0.3) and RVEDV decreased from 157.3±42.3cm³ to 154.6±41.3cm³ (P=0.4). These
differences were not significant between the groups over the 12 study duration (group x time interaction; LVEDV P=0.6, RVEDV P=0.2).

Myocardial mass increased from 138.3±38.1g to 140.7±37.7g (P=0.05) in the control group, whereas the intervention group showed a reduction from 137.6±28.2g to 123.1±25.3g (P<0.001) and the group x time significance was P=0.03. In addition, MM indexed to height increased from 79.1±19.5gm⁻¹ to 80.5±19.3gm⁻¹ in the control group and decreased from 77.9±14.8gm⁻¹ to 69.7±13.6gm⁻¹. The group x time differences in myocardial mass indexed to height remained significant over the follow-up duration, P=0.01.

**Pericardial fat and AF burden**

A significant correlation was observed between the change in PF volume and the change in; weight (r=0.30, P=0.048), SBP (r=0.31, P=0.025), HOMA-IR (r=0.33, P=0.043), LA volume (r=0.48, P<0.001), RA volume (r=0.64, P<0.001) and MM (r=0.62, P<0.001). However, only a trend was observed between the change in PF volume and the change in WC (r=0.25, P=0.08), and no association observed for the change in BMI, BSA, CRP and DBP. There was a significant correlation between the reduction in PF and the reduction in AF frequency (r=0.49, P=0.003), AF duration (r=0.40, P=0.017) and AF severity (r=0.47, P=0.004).

For AF frequency, the following variables were entered into the regression model (P≤0.2); HOMA-IR, SBP, maximum LA volume, MM, BMI, WC, PF and BSA. Only PF was a significant predictor of AF frequency, \( r^2=0.24, P=0.003 \). For AF duration, the same variables, except HOMA-IR, were entered into the regression model (P≤0.2) and only PF was the significant predictor of AF duration, \( r^2=0.16, P=0.017 \). For AF severity; BMI, maximal LA volume, MM,
PF and BSA were entered into the regression model (P≤0.2), however only PF was predictive of AF severity, \( r^2=0.22, P=0.004 \) (FIGURE3).

Discussion

Main findings

In this prospective randomized and controlled study, we enrolled patients in either an interventional weight reduction or a general advice (control) group, and determined the PF volume, cardiac structural changes and AF severity over 12 months follow-up. Our study findings show that weight reduction is associated with;

1. A significant reduction in the PF depot.
2. A decline in maximal and minimal LA and RA volumes. Furthermore, this observation remained significant for height-adjusted measurements.
3. An improvement in blood pressure and ventricular mass, which again persisted following indexing to height

In both groups, there appeared to be a uniform reduction in insulin resistance and systemic inflammation, however the differences between the groups was not significant.

An important and novel finding in our study is the favorable effect of weight reduction on AF severity, and its relationship to the PF depot. The decline in PF volume was a stronger predictor of the decline in parameters of AF burden, as compared to the decline in BMI, WC and BSA. This observation supports the inference that PF may represent a pathophysiological intermediary between atrial remodeling and the arrhythmogenic substrate, in overweight and obese patients. This association may reflect the contiguous
nature of both structures and therefore raising the possibility of a paracrine mechanism as had been observed in other disease states\cite{268}.

*Obesity and pericardial fat*

Pericardial fat has been shown to reflect traditional measures of obesity such as BMI, visceral adipose tissue on computed tomography, waist circumference as well as markers of low grade systemic inflammation\cite{258, 269}. Monozygotic twin studies have suggested that the PF depot is primarily modulated by environmental influences, rather than genetic factors\cite{270}.

Snel and colleagues recently demonstrated the amenability of this depot to dietary modulation through a VLCD program, and persistence of benefit despite body weight and abdominal visceral fat re-gain in the longer term\cite{271}. On the other hand Nakazato and colleagues have shown epicardial fat as quantified by abdominal CT closely reflects changes in both weight loss and weight gain\cite{272}. In our cohort, we observed a 15% mean reduction in PF volume with a corresponding 15% reduction in total body weight. These observations collectively suggest that PF may be at least partially under systemic control during weight loss.

*Pericardial fat and atrial fibrillation*

Pericardial fat has been recognized as a novel risk factor for incident AF, its chronicity and severity\cite{91}. In addition, a high PF burden has been shown to predict worse AF severity as assessed by the AFSS, greater chronicity and worse catheter ablation outcomes\cite{93}. In our study we demonstrated a significant association between AF burden and PF volume and that PF was strongly predictive of the reduction in each parameter of AF burden.

*Atrial remodeling, obesity and atrial fibrillation*
Atrial structural and conduction changes have been studied in various conditions associated with obesity. The presence of tissue structural changes, low myocardial voltages and frank scar has been shown to accompany the observed conduction slowing and dispersion\textsuperscript{28,67,224}. In addition obese patients have been shown to have LA hypertension and dilation and this is associated with shortening of atrial myocardial and pulmonary vein tissue refractoriness\textsuperscript{193}. Moreover, the presence of LA enlargement has an adverse effect on AF progression and therapeutic outcomes and may be amenable to reverse remodeling\textsuperscript{273-275}. The current study shows that weight reduction has a favorable effect on atrial size and the PF depot.

**Limitations**

This study employed a prospective and randomized design and utilized cardiac MRI to accurately quantify cardiac chamber volumes and a reproducible method for quantifying pericardial fat. Nevertheless, firstly, the study was conducted primarily in a male Caucasian population, hence limiting interpretability in other patient groups. Secondly, we cannot exclude the possibility that the change in pericardial fat depot is an epiphenomenon of weight loss and is unrelated to the favorable changes in atrial volumes. Finally, a non-invasive quantification of AF burden may underestimate the true arrhythmia burden.

**Conclusion**

A physician delivered weight management program results in a reduction in PF volumes, indexed atrial volumes and myocardial mass. This occurred in conjunction with improved blood pressure, systemic markers of the metabolic syndrome and indices of AF severity.
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</tr>
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<td>Hyperlipidemia n (%)</td>
<td>23 (70)</td>
<td>20 (56)</td>
</tr>
<tr>
<td>Coronary artery disease n (%)</td>
<td>5 (15)</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Valve disease n (%)</td>
<td>0</td>
<td>2 (6)</td>
</tr>
<tr>
<td>Excess alcohol consumption n (%)</td>
<td>13 (39)</td>
<td>12 (33)</td>
</tr>
<tr>
<td>Tobacco use n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>2 (6)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Never</td>
<td>19 (58)</td>
<td>23 (64)</td>
</tr>
<tr>
<td>Reformed</td>
<td>12 (36)</td>
<td>12 (33)</td>
</tr>
<tr>
<td>Sleep apnea n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>4 (13)</td>
<td>5 (16)</td>
</tr>
<tr>
<td>Mild</td>
<td>11 (35)</td>
<td>11 (34)</td>
</tr>
<tr>
<td>Moderate</td>
<td>7 (23)</td>
<td>10 (31)</td>
</tr>
<tr>
<td>Severe</td>
<td>9 (29)</td>
<td>6 (19)</td>
</tr>
<tr>
<td>CPAP use</td>
<td>8 (24)</td>
<td>8 (23)</td>
</tr>
<tr>
<td>RDI</td>
<td>22.3±14.7</td>
<td>23.2±13.5</td>
</tr>
</tbody>
</table>
TABLE 2: Metabolic and AF severity (AFSS) parameters.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12 months</th>
<th>P value</th>
<th>Control group (n=33)</th>
<th>Intervention group (n=36)</th>
<th>P value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>134±18</td>
<td>134±14</td>
<td>1.0</td>
<td>133±17</td>
<td>130±13</td>
<td>0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82±12</td>
<td>81±8</td>
<td>0.8</td>
<td>81±11</td>
<td>79±8</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>HOMA-IR (units)</td>
<td>8.3±3.42</td>
<td>9.6±4.27</td>
<td>0.2</td>
<td>4.5±2.85</td>
<td>3.8±2.54</td>
<td>0.4</td>
<td>0.07</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>2.3±1.36</td>
<td>2.2±1.77</td>
<td>0.9</td>
<td>1.7±1.11</td>
<td>1.3±0.98</td>
<td>0.2</td>
<td>0.09</td>
</tr>
<tr>
<td>AFSS – Frequency</td>
<td>7.1±2.0</td>
<td>7.1±1.5</td>
<td>0.9</td>
<td>6.8±2.5</td>
<td>3.2±1.2</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AFSS – Duration</td>
<td>7.6±2.3</td>
<td>6.9±2.2</td>
<td>0.2</td>
<td>6.6±3.0</td>
<td>2.1±1.6</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AFSS – Severity</td>
<td>6.8±1.8</td>
<td>6.8±2.4</td>
<td>0.9</td>
<td>5.7±2.5</td>
<td>2.8±1.5</td>
<td>&lt;0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>AFSS – Total</td>
<td>21.4±4.8</td>
<td>20.8±4.1</td>
<td>0.6</td>
<td>19.0±7.4</td>
<td>8.1±3.5</td>
<td>&lt;0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>AFSS – Symptom</td>
<td>15.9±6.9</td>
<td>16.4±7.4</td>
<td>0.8</td>
<td>14.4±8.0</td>
<td>6.1±4.1</td>
<td>&lt;0.001</td>
<td>0.003</td>
</tr>
</tbody>
</table>
**TABLE 3:** Cardiac MRI changes.

<table>
<thead>
<tr>
<th></th>
<th>Baseline Control group (n=33)</th>
<th>12 months Intervention group (n=36)</th>
<th>P value</th>
<th>Baseline Control group (n=33)</th>
<th>12 months Intervention group (n=36)</th>
<th>P value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV EDV (mL)</td>
<td>167 (133-193)</td>
<td>167 (133-199)</td>
<td>0.7</td>
<td>166 (128-197)</td>
<td>167 (134-192)</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>LV ESV (mL)</td>
<td>49 (38-71)</td>
<td>44 (33-62)</td>
<td>0.2</td>
<td>47 (37-68)</td>
<td>43 (35-55)</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>RV EDV (mL)</td>
<td>148 (106-172)</td>
<td>150 (130-187)</td>
<td>0.2</td>
<td>152 (105-172)</td>
<td>147 (128-182)</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>RV ESV (mL)</td>
<td>52 (42-77)</td>
<td>54 (41-73)</td>
<td>0.9</td>
<td>48 (42-73)</td>
<td>55 (40-69)</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Myocardial mass (g)</td>
<td>136 (113-162)</td>
<td>134 (118-165)</td>
<td>0.9</td>
<td>143 (118-164)</td>
<td>117 (107-142)</td>
<td>0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Myocardial mass indexed (gm$^{-1}$)</td>
<td>78 (65-92)</td>
<td>76 (68-88)</td>
<td>0.8</td>
<td>82 (66-91)</td>
<td>66 (61-76)</td>
<td>0.009</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LA volume (max) (mL)</td>
<td>111 (85-128)</td>
<td>105 (91-119)</td>
<td>0.5</td>
<td>112 (86-128)</td>
<td>98 (88-105)</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LA volume (max) indexed (mLm$^{-1}$)</td>
<td>62 (50-72)</td>
<td>60 (52-65)</td>
<td>0.3</td>
<td>62 (50-70)</td>
<td>55 (50-60)</td>
<td>0.005</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LA volume (min) (mL)</td>
<td>68 (51-93)</td>
<td>62 (48-84)</td>
<td>0.2</td>
<td>67 (51-93)</td>
<td>60 (47-71)</td>
<td>0.01</td>
<td>0.004</td>
</tr>
<tr>
<td>LA volume (min) indexed (mLm$^{-1}$)</td>
<td>38 (31-51)</td>
<td>35 (28-46)</td>
<td>0.1</td>
<td>38 (31-51)</td>
<td>34 (27-40)</td>
<td>0.009</td>
<td>0.003</td>
</tr>
<tr>
<td>RA volume (max) (mL)</td>
<td>98 (77-115)</td>
<td>98 (88-105)</td>
<td>0.9</td>
<td>100 (78-112)</td>
<td>83 (75-97)</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RA volume (max) indexed (mLm$^{-1}$)</td>
<td>58 (47-62)</td>
<td>58 (47-62)</td>
<td>0.8</td>
<td>57 (47-62)</td>
<td>48 (43-56)</td>
<td>0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RA volume (min) (mL)</td>
<td>62 (47-82)</td>
<td>55 (41-75)</td>
<td>0.5</td>
<td>62 (47-82)</td>
<td>52 (40-62)</td>
<td>0.06</td>
<td>0.007</td>
</tr>
<tr>
<td>RA volume (min) indexed (mLm$^{-1}$)</td>
<td>34 (28-47)</td>
<td>30 (24-41)</td>
<td>0.3</td>
<td>33 (27-47)</td>
<td>29 (23-35)</td>
<td>0.03</td>
<td>0.007</td>
</tr>
<tr>
<td>Pericardial adipose tissue (cm$^{3}$)</td>
<td>130 (106-184)</td>
<td>136 (119-171)</td>
<td>0.8</td>
<td>128 (107-189)</td>
<td>115 (93-134)</td>
<td>0.007</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Figure legend

Figure 1: Patient flow and retention.

Figure 2: Changes in waist circumference, weight and BMI between the study groups over the duration of follow-up. At 3 months; BMI P=0.01. At 6 months; BMI P=0.005. At 9 months; WC P=0.007, weight P=0.02, BMI P<0.001. At 12 months; WC P=0.004, weight P=0.008, BMI P=0.001.

Figure 3: Multivariable analysis showing the regression coefficient forest plots for the variables predictive of each AFSS domain; frequency (top panel), duration (middle panel) and episode severity (lower panel).
FIGURE 1: Patient flow.

178 patients screened for eligibility

103 patients recruited and randomized

75 declined participation

34 excluded:
16 underwent intra cardiac metallic implant
1 declined second MRI scan
10 declined further participation
7 poor images

69 patients available for analysis
• 33 Control
• 36 Intervention
FIGURE 2: Changes in waist circumference, weight and body mass index.
FIGURE 3: Predictors of AF burden.
Supplement:

NOTE:
This supplement is included on pages 164-165 of the print copy of the thesis held in the University of Adelaide Library.
Chapter 5

Summary and Final Discussion

In summary, this thesis presents a body of work investigating the mechanistic relationship between AF and overweight/obesity, and its amenability for non-invasive clinical intervention to ameliorate the severity of the syndrome-disease complex.

Chapter 1 addresses the enormity of the problem and the landmark epidemiological studies implicating obesity as a significant risk factor for AF. The role of several obesity-related co-morbidities or frequently associated conditions such as hypertension, coronary artery disease, sleep disordered breathing, cardiac failure, hyperinsulinemia and diabetes mellitus and excess alcohol consumption is discussed. Left atrial disease is identified as the common “substrate” leading to atrial fibrillation as a result of obesity, and also for the other aforementioned risk factors. The potential roles of lipid infiltration, mediators produced by local and remote adipose tissue stores, inflammation, increased left atrial size and wall tension, dysregulated myocardial energetics and cellular fuel utilization subsequent to dietary manipulation and hyperinsulinemia, are examined.

To investigate the electrophysiological and structural basis of atrial remodeling, we described the landmark human and animal studies, in addition to their limitations. The cellular structural changes, functional-electrical changes, the ionic current basis, centrality of calcium conductance and the time course of the hallmark events marking the remodeling process are discussed. In addition to functional and structural changes, the relevant candidate molecular mechanisms identified in the atrial myocardial fibrotic process and the potential relationship to overweight/obesity are described.
Following discussion of the remodeling process and the mechanisms promoting it, we discuss the clinical implications for therapeutic options in the management of AF. The first scientific paper explores the effect of progressive weight gain on atrial structure and electrical function. This preclinical work utilizes an ovine (sheep) model reflective of human obesity (excess calorie-dense food intake) to induce weight gain and accompanying well-described hemodynamic disturbances of systemic and left atrial hypertension. In addition to quantifying the hemodynamic disturbances, cardiac MRI is used to determine progressive structural changes in cardiac chambers and the ectopic pericardial fat burden. This work demonstrates the novel finding of an obesity-related atrial substrate promoting arrhythmogenesis. In this model, obesity was associated with increased pericardial fat volume, bi-atrial volumes and myocardial mass on cardiac MRI. A gradual and early slowing in conduction velocity was largely uniform throughout the atrial muscle (appendage or free wall) and was accompanied by an increased dispersion of conduction velocities (conduction heterogeneity). Statistical adjustment for the observed progressive left atrial and systemic hypertension showed the persistence of these tissue electrical abnormalities. Furthermore, these tissue electrical abnormalities were observed to be partially functional in nature; rapid pacing amplified the conduction disturbances and the addition of a premature extra stimulus (simulating ectopic triggers for reentry) also exaggerated these conduction abnormalities. Interestingly, there was no significant change in the effective refractory period. The number of spontaneous and induced AF episodes (>30 seconds) as well as the duration of the episodes, increased exponentially with increasing weight. At an ultra-structural level, it was noted that early in the weight gain trajectory; there was intramyocardial tissue lipid infiltration. Later in the weight gain trajectory inflammatory cell
infiltration and collagen deposition were evident. The role of intra-myocellular lipid accumulation and changes in intermediary metabolism and energetics requires further investigation. There was evidence of a significant increase in the expression of Endothelin-A and Endothelin-B receptor proteins on atrial myocardium accompanied by a trend towards an increased cytoplasmic Endothelin-1 ligand peptide. In the context of the current literature, the Endothelin system has been identified as an important contributor to the pathophysiology of cardiac structural and conduction changes. This may represent an avenue for further proof-of-concept mechanistic studies using pharmacological manipulation. Additionally, increased expression of PDGF expression was identified. PDGF has been reported to have a pathophysiologic role in the development of atrial fibrosis particularly in the context of hemodynamic disturbances affecting the left atrial chamber.

Concurrently, there was an increased expression of TGF-β1. This molecule has been demonstrated to play a critical role in the regulation of cardiac fibroblasts. Finally, there was a small trend in the change in CTGF with increasing weight. Studies have demonstrated the important role of CTGF in mediating the atrial fibrotic process, using animal models of AF. Our observation of a modest trend between CTGF and weight warrants further studies elucidating its specific role in atrial changes consequent to weight gain and obesity.

Therefore in summary, this large animal preclinical model demonstrates that progressive weight results in:

1. Specific tissue electrical and mechanical changes
2. Hemodynamic disturbances which contribute only partially in promoting these conduction abnormalities
3. Tissue changes characterized by fibrosis, inflammation and lipidosis. The latter is congruent with the change in the increased pericardial ectopic fat burden

4. Increased pro-fibrotic mediators, with the principal novel finding of abnormal Endothelin system signaling

A plausible explanation is that beyond the well-established hemodynamic disturbances promoting an electro-structural substrate, the local pericardial fat depot may promote a local, and possibly systemic, tissue injury and abnormal myocardial energetics. The latter changes require further mechanistic studies.

The feasibility of the reversal of the AF substrate is a topic which has received much attention in the literature recently. The identification and characterization of the various epidemiological risk factors for AF has raised the question of whether directed intervention at these, may ameliorate the burden, epidemiological and individual, of the arrhythmia.

Hypertension is traditionally recognized as the most significant epidemiological risk factor for developing AF. Interventional studies focusing on aggressive management of hypertension have demonstrated a favorable change in left atrial size, and therefore reducing the risk of new onset AF. On the other hand, mechanistic studies have shown that relief of left atrial hypertension following mitral valve surgery for mitral stenosis, results in a reversal of the electro-structural markers promoting AF. In this translational study, an overweight and obese cohort of patients with symptomatic AF was prospectively recruited and studied. The effect of a physician-directed interventional weight management program, in conjunction with strict cardio-metabolic risk factor identification and optimal management was compared to a control group receiving general lifestyle advice and likewise strict cardio-metabolic risk factor management, in a randomized fashion.
Additionally, this latter group received marine triglyceride supplementation. For ethical reasons, the control group needed to have cardio-metabolic risk factors managed in the same strict fashion as in the intervention group, however with weight reduction being a largely self-directed process, coupled with less frequent physician follow-up, control of these risk factors was inherently less efficacious. Cardio-metabolic risk factors managed were; sleep apnea, hypertension, hyperlipidemia, smoking, unfavorable alcohol consumption, glucose intolerance and diabetes mellitus. Weight reduction was undertaken in the intervention group using a previously described process employing a modified VLCD for one month, followed by a gradual employment of measures pertinent to permanent lifestyle change and sustained weight reduction. Patients in both groups were periodically assessed; however the intervention group had access to greater physician contact and support to maintain a favorable weight loss trajectory. To determine the burden of AF, the validated semi-quantitative questionnaire, AFSS, was administered in addition to 7-day continuous ambulatory cardiac rhythm recording. Cardiac structure was assessed using transthoracic echocardiography due to ease of use and capacity for wider clinical monitoring applicability. At 12 months follow-up, there was a divergence in the weight and BMI between both groups. The AFSS sub-scores of AF frequency, duration, episode severity and symptom severity all declined to a greater extent in the intervention group, relative to the control group. It is interesting to note that the control group, which experienced a lesser but significant weight reduction, and likewise cardio-metabolic risk factor control, demonstrated a reduction in AFSS-quantified AF burden. This emphasizes the benefit of risk factor control in positively influencing AF burden, and the greater favorable effect of weight loss, over and above risk factor control alone.
The group difference in AF burden was also confirmed on 7 day ambulatory cardiac rhythm monitoring. Furthermore, the intervention group, relative to controls, required less anti-arrhythmic medication to achieve this reduction in AF burden and less anti-hypertensive agents to achieve target levels of blood pressure. In addition, the intervention group demonstrated a reduction in LA size and measures of left ventricular hypertrophy. Taken together, these findings suggest a more effective control of cardio-metabolic risk factors and arrhythmia burden as a result of weight reduction, and that this effect was associated with favorable cardiac reverse remodeling.

The finding of this study exemplifies the opportunity to alter the course of this arrhythmia, which appears to largely reflect an underlying deranged cardio-metabolic state. This may, in addition to curbing the severity of obesity-associated conditions, have a favorable effect on the arrhythmia burden and symptomatology.

To further explore novel mechanisms implicated in the obesity-AF relationship, an imaging study was performed to study the impact of weight reduction on the local pericardial fat depot. Eligible patients recruited for the study of weight and risk factor management on the burden of AF were offered the additional study component of cardiac MRI imaging. Of the patients recruited, 69 completed baseline and follow-up imaging scans. The primary reasons cited by subjects for withdrawal was claustrophobia. Nevertheless, the groups were well randomized at baseline with respect to anthropometric measures. The study was therefore prospective, randomized and controlled. The study outcomes were changes in pericardial fat volume, cardiac structure, cardio-metabolic risk markers and the semi-quantitative assessment of AF burden over 12 months follow-up. Post-hoc analysis was performed to study the predictive effect of each risk marker and the reduction in semi-quantitative AF
burden. There was a reduction in AF burden similar to the observation made in chapter 3 concurrent with an improvement in cardio-metabolic markers, atrial size, myocardial mass and pericardial fat volume. On multiple regression analysis, of all clinically relevant predictors of the reduction in AF burden, the change in pericardial fat volume was the strongest. The results of this study suggest an independent influence of the pericardial fat depot on AF burden and severity. Although limited by small numbers and more than 30% lost to follow-up, this observation strengthens the plausibility of the independent effect of the pericardial fat depot in influencing the subtended atrial myocardium, and promoting an AF substrate. Therefore, in addition to the systemic impact of overweight and obesity, a local critical effect may be attributable to the pericardial fat depot and this is amenable to lifestyle and dietary intervention.

In summary therefore, this body of work illustrates the effect of obesity on atrial remodeling and promoting the AF substrate, both through local and remote mechanisms. Importantly, our work illustrates the amenability to influence the severity of AF through management of the underlying metabolic disturbances, particularly weight loss for overweight and obesity.

**Future Directions**

*Pathophysiologic preclinical mechanisms and reversibility*

The obese ovine model studied in chapter 1 has described a weight-gain associated atrial myocardial electrical, structural and functional abnormality predisposing to AF. To further define this mechanism several steps need to be further evaluated.
In chapter 3, we observed a reversibility of the AF burden and severity with interventions to manage obesity and cardio-metabolic risk factors. The specific electrophysiological and biochemical pathways underlying this reversibility require further studies. The biochemical perturbations associated with progressive weight gain and their dynamic changes with weight loss, such as glucose and lipid changes, serum metabolic cytokines and their receptors and markers of systemic inflammation need to be evaluated and their potential contributory role to the observed tissue abnormality defined.

In chapter 2, several putative biochemical mediators predisposing to cardiac fibrosis were identified. Causality through pharmacological manipulation and microarray studies to define the upstream and downstream components would clarify areas amenable to therapeutic targeting. Manipulation using anti-ligand or anti-receptor antibodies in the obese model may elucidate the specific role of each molecule in the fibrosis cascade. Importantly, Endothelin system interference would clarify the role of this pathway in this disease syndrome complex and therefore providing avenues for potential pharmacotherapeutic targeting.

Clinical intervention

This clinical study was conducted as a single center study with 12 months of follow-up. There was a significant loss of study subjects by 12 months (~30%). Although compatible with most weight loss studies, patient retention remains as one of the challenges in weight loss. This is critical for the study power and reliability of the results. In addition, a multi-collaborative, multi-center study with a larger patient cohort, longer follow-up duration and a more reliable AF quantification method is required. Long-term weight maintenance
following successful weight reduction is a significant challenge in the clinical and research arenas. In addition, the ideal method for quantifying AF episodes requires the invasive implantation of a monitoring device. Therefore, a study which investigates the on-going weight trajectory, beyond 12 months, and whether this translated to sustained benefit in terms of AF burden, is required. In addition, the feasibility to accurately quantify AF burden is possible in the near future, with the rapid evolution of continuous ambulatory cardiac rhythm monitoring devices. Moreover, elucidation of the role of sleep apnea would require a standardized CPAP commencement time and a repeat sleep study at follow-up. With such additional information, a multivariable model may be constructed to compare the relative strength of each risk factor intervention component in predicting favorable outcomes for AF. The clinical study may also have limited applicability to non-Caucasian populations, given the demographic from which subjects were recruited. By extension, other recruitment issues may be rectified by age and gender stratified randomization over multiple centers with greater subject numbers, allowing enough power for multivariable post-hoc and subgroup analysis.

In terms of program delivery, this study presents an efficacious method to implement weight reduction and cardio-metabolic risk factor management in a high risk group of patients. This may be delivered with a low level of acceptable adverse events. The weight management program may be integrated into the ambulatory care of patients with AF in primary care or specialist clinics, with specifically-trained multidisciplinary personnel. A pilot study with cost effectiveness evaluation is warranted, to encourage wider applicability. Finally, to integrate the findings in this body of work, a multifaceted approach employing weight reduction, cardio-metabolic risk evaluation and management in addition to pro-
fibrotic pathway interference, such as the use of Endothelin antagonists, requires further study.
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