ATRIAL FIBRILLATION AND SUDDEN CARDIAC DEATH IN OBESITY: AN INVESTIGATION OF THE ARRHYTHMOGENICITY OF EPICARDIAL FAT

By

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To My Parents Mr. Adam and Mrs. Mary R. Agbaedeng

ABSTRACT

Atrial fibrillation and sudden cardiac death are two burgeoning cardiac disorders caused by arrhythmic events in the heart. Patients with atrial fibrillation are at an increased risk of severe cardiovascular complications, hospitalisation, thromboembolic events, clinical morbidity, and mortality. Premature death resulting from sudden cardiac death is a significant cause of cardiovascular mortality, often occurring in patients without apparent high-risk conditions and with normal heart functions. The emergence of obesity epidemic in the community is implicated in the rising burdens of atrial fibrillation and sudden cardiac death, but this has not been well characterised. More recently, the ectopic cardiac fat depot "epicardial adipose tissue" is postulated to mediate the pro-arrhythmic sequalae of obesity. In this thesis, investigations were undertaken to characterise these relations in a meta-analysis; and to evaluate the cardiac electrophysiological and structural substrates due to epicardial fat in ovine sheep models of chronic weight gain and weight fluctuations.

In chapter 2, a comprehensive systematic review of the literature and a meta-analysis were conducted to define the association of the fibrotic biomarker galectin-3 and atrial fibrillation. The findings demonstrated significant associations of high serum galectin-3 and risk and severity of atrial fibrillation.

In chapter 3, a comprehensive systematic review of the literature and a meta-analysis were conducted to define the clinical associations of epicardial fat and atrial fibrillation, arrhythmia progression, recurrent atrial fibrillation following curative catheter ablation, and post-operative atrial fibrillation after cardiac surgery. The findings demonstrated significant associations of increased expansions of total cardiac and peri-atrial epicardial adipose tissue

with greater risk of atrial fibrillation; severity of atrial fibrillation; atrial fibrillation recurrence post-ablation; and *de novo* incidence after cardiac surgery.

Next, the underlying mechanisms were explored in chronic ovine models and presented in **chapters 4 & 5**. The results demonstrated that obesity induces expansion of epicardial fat and fibro-fatty replacement of atrial myocytes and deterioration of myocyte contractile apparatus, which may drive impairments of atrial electrical properties. Despite having comparable epicardial fat quantity with reference controls, weight fluctuation, induced similar abnormalities, albeit less severe, with stable obesity, thus highlighting an explanation for the increased atrial arrhythmias risks often seen with periodic fluxes in weight.

Chapter 6 reports findings from a systematic review and meta-analysis undertaken to define the association between obesity and sudden cardiac death. The pooled analyses involving over 1.4 million patients demonstrated that, after correcting for traditional high-risk risk factors: underweight body mass index (<18.5 kg.m^{-2}) associates with an increased risk of sudden cardiac death; overweight shows no significant association with sudden cardiac death; obesity (BMI: ≥ 30 kg.m^{-2}) predicts an exaggerated risk for sudden cardiac death. Similarly, unit increment in body mass index was shown to demonstrate a greater risk for sudden cardiac death, further implicating the role of increased adiposity in the risk of sudden cardiac death.

In chapter 7, the molecular and structural substrates for ventricular arrhythmias that lead to sudden cardiac death in a model of chronic obesity are presented. Obesity demonstrated two-and-half-fold expanded ventricular epicardial fat depot with a consequent extensive and severe fat cell infiltrations; significant reduction in ventricular desmosomal cadherin desmoglein-2, which demonstrated significant negative correlation with the degree

of fatty infiltration; and induction of diffuse ventricular interstitial fibrosis. The findings further demonstrated that obesity results in significant abnormal modulation of fibrotic pathways, including an alternative component of the central transforming growth factor-beta 1 pathway, angiotensin II, endothelin and aldosterone signalling pathways.

The observations of epicardial fat expansion and subsequent fibro-fatty infiltrations are particularly noteworthy. Epicardial fat adds an important extra layer to the stratification of patients at risk of atrial fibrillation and sudden cardiac death. Fibro-fatty infiltrates alone are sufficient to induce re-entrant tachyarrhythmias, leading to atrial fibrillation and sudden cardiac death. Clinical assessment of fibro-fatty infiltrates could help improve sudden cardiac death risk profiling of patients in the low-risk communities, who paradoxically have high absolute mortality rates. More importantly, the fibro-fatty deposits could form a key element in substrate mapping as a guide for ablation of lethal arrhythmias.

DECLARATIONS

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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 in my name, 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.

I certify that the writing of this thesis, the results, interpretations, opinions, and suggestions are entirely my own creation. This thesis does not exceed the length of 80,000 words inclusive of tables, figures and figure legends, and bibliographic references.

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Publications, Presentations and Prizes

Chapter 1:

Manuscripts

1. Agbaedeng TA, Linz D, Lau DH, Sanders P. Unique role of epicardial adipose tissue in atrial fibrosis – 'Atrial remodeling of a new sort.' *Heart Rhythm* 2018;15(11):1728-1729
2. Mahajan R, Nelson A, Pathak RK, Middeldorp ME, Wong CX, Twomey DJ, Carbone A, Teo K, Agbaedeng T, Linz D, de Groot JR, Kalman JM, Lau DH, Sanders P. Electroanatomical remodeling of the atria in obesity – impact of adjacent epicardial fat. *Journal of American College of Cardiology: Clinical Electrophysiology* 2018; 4(12): 1529-1540. doi: 10.1016/j.jacep.2018.08.014
3. Thanigaimani S, McLennan E, Linz D, Mahajan R, Agbaedeng TA, Lee G, Kalman JM, Sanders P, Lau DH. Progression and reversibility of stretch induced atrial remodeling – characterization and clinical implications. *Progress in Biophysics and Molecular Biology* 2017;130(Pt B):376-386. doi: 10.1016/j.pbiomolbio.2017.07.010
4. Thanigaimani S, Lau DH, Agbaedeng T, Elliott AD, Mahajan R, Sanders P. Molecular mechanisms of atrial fibrosis – implications for the clinic. *Expert Reviews in Cardiovascular Therapy* 2017;15(4):247-256. doi: 10.1080/14779072.2017.1299005

Chapter 2:

Agbaedeng TA, Munawar DA, Rattanakosit T, Kadhim IK, Elliott AD, Linz D, Hendriks J, Mahajan R, Lau DH, Sanders P. Galectin-3 as a predictor of atrial fibrillation – a meta-analysis. **UNDER REVIEW**

Presentations

1. Presented as a Poster: The European Heart Rhythm Association Congress, Lisbon, March 2019

   Abstract: TA. Agbaedeng, DA. Munawar, M. Emami, R. Mahajan, DH. Lau, P. Sanders. Independent association of galectin-3 with risk of atrial fibrillation. *EP Europace* 2019;21(supplement_2)
2. Presented as a Poster: The 11th Asia Pacific Heart Rhythm Society Scientific Session; Taipei, Taiwan, October 2018

   Abstract: Agbaedeng TA, Emami M, Munawar DA, Elliott A, Linz D, Mahajan R, Lau DH, Sanders P. Galectin-3 as an independent risk marker of atrial fibrillation – a systematic review and meta-analysis. *Journal of Arrhythmia* 2018
3. Presented as a Poster: The 64th Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand, Brisbane, August 2018

   Abstract: Agbaedeng TA, Munawar DA, Elliott A, Linz D, Mahajan R, Lau DH, Sanders P. Galectin-3 and atrial fibrillation – prognostic utility of a pro-fibrotic biomarker. *Heart, Lung and Circulation* 2018;27(supplement_2):S162–S163

Prizes

National Scientific Meeting Travel Award, International Society for Heart Research – Brisbane, 2018

Chapter 3:

Manuscript

Agbaedeng TA, Mahajan R, Munawar DA, Elliott AD, Twomey DJ, Linz D, Hendriks J, de Groot JR, Lau DH, Sanders P. Epicardial adipose tissue and atrial fibrillation risk – a systematic review and meta-analysis. **UNDER REVIEW**

Presentation

1. Oral Presentation: The 63rd Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand; Adelaide, AUSTRALIA, August 2016.
   Abstract: Agbaedeng TA, Mahajan R, Munawar DA, Elliott A, Twomey DJ, Kumar S, Lau DH, Sanders P. Meta-analysis of effects of epicardial fat on atrial fibrillation and ablation outcome. *Heart, Lung and Circulation* 2016;25(2):S150
2. Presented as a Poster: The 63rd Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand; Adelaide, AUSTRALIA, August 2016
   Abstract: Agbaedeng TA, Mahajan R, Munawar DA, Elliott A, Twomey DJ, Kumar S, Varzaly J, Gallagher C, Lau DH, Sanders P. Effects of epicardial fat and anterior fat pad removal on post-operative atrial fibrillation – a systematic review and meta-analysis. *Heart, Lung and Circulation* 2016;25(2):S292
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Abstract: Agbaedeng TA, Mahajan R, Munawar DA, Elliott A, Twomey DJ, Kumar S, Varzaly J, Gallagher C, Lau DH, Sanders P. Effects of epicardial fat and anterior fat pad removal on post-operative atrial fibrillation – a systematic review and meta-analysis. *European Heart Journal* 2016;37:S884

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   Abstract: Agbaedeng TA, Mahajan R, Munawar DA, Elliott A, Twomey DJ, Pathak RK, Kumar S, Lau DH, Sanders P. The association between epicardial fat and atrial fibrillation – a systematic review and meta-analysis of the increasing evidence. *Journal of Arrhythmia* 2015

5. Presented as a Poster: The 8th Asia Pacific Heart Rhythm Society Scientific Session; Melbourne, AUSTRALIA, November 2015

   Abstract: Agbaedeng TA, Mahajan R, Munawar DA, Elliott A, Twomey DJ, Pathak RK, Kumar S, Lau DH, Sanders P. Association between epicardial fat and post-operative atrial fibrillation – a systematic review and meta-analysis. *Journal Arrhythmia* 2015

Chapter 4&5:

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1. Agbaedeng TA, Twomey DJ, Thanigaimani S, Manavis J, Verjan J, Franke K, Kuchel TR, Linz D, Lau DH, Mahajan R, Sanders P. Electrical and electroanatomic characterisation of the atria in obesity and weight fluctuation. **UNDER REVIEW**

2. Agbaedeng TA, Twomey DJ, Thanigaimani S, Manavis J, Verjan J, Franke K, Kuchel TR, Linz D, Lau DH, Mahajan R, Sanders P. Cellular mechanisms of epicardial fat in obesity and weight fluctuation - fibrofatty infiltrations, myofibrillar remodelling and lipid imaging. **UNDER REVIEW**

Presentations

1. To be presented for the Young Investigator Award: The 65th Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand | International Society for Heart Research, Adelaide, August 2019

   Abstract: T. Agbaedeng, D. Twomey, S. Thanigaimani, D. Linz, D. Lau, R. Mahajan, P. Sanders. Weight fluctuation induces formation of pro-arrhythmic substrate by fibro-fatty depositions and residual electro-structural remodelling: evidence from an ovine model. *Heart Lung and Circulation* 2019; DOI: https://doi.org/10.1016/j.hlc.2019.06.037 (**In Press**)
2. To be Presented as a Moderated Poster: The European Society of Cardiology 2019 Congress, Paris, France, August/September 2019

   Abstract: Agbaedeng TA, Twomey DJ, Thanigaimani S, Linz D, Lau DH, Mahajan R, Sanders P. Weight fluctuation demonstrates residual atrial arrhythmogenic substrate despite final weight loss in a chronic sheep model – implications for epicardial fat and fibrofatty infiltrates. *European Heart Journal* 2019
3. Presented as a 15-minute Oral Presentation: The 23rd International Society for Heart Research World Congress, Beijing, June 2019

   Abstract: Agbaedeng TA, Twomey DJ, Thanigaimani S, Linz D, Lau DH, Mahajan R, Sanders P. Epicardial fat and fibro-fatty deposits promote atrial substrate in

chronic sheep models – implication for sustained weight gain and fluxes during weight loss. *Journal of Molecular and Cellular Cardiology* 2019

4. Presented as a Poster: The 40th Annual Scientific Sessions of the Heart Rhythm Society, San Francisco, May 2019

   Abstract: Agbaedeng TA, Twomey DJ, Thanigaimani S, Elliott AD, Linz D, Lau DH, Mahajan R, Sanders P. Atrial electro-structural substrates in stable obesity and weight fluctuation in ovine models – epicardial fat and fibro-fatty cardiomyopathy as potential mediators. *Heart Rhythm* 2019

Prizes

CSANZ Travelling Fellowship to the European Society of Cardiology, the Cardiac Society of Australia and New Zealand – Sydney, 2019

Chapter 6:

Manuscripts

Agbaedeng TA, Mahajan R, Munawar DA, Khokhar KK, Twomey DJ, Middeldorp M, Gallagher C, Elliott AD, Hendriks J, Linz D, Lau DH, Sanders P. Obesity and sudden cardiac death – a meta-analysis of 1.4 million individuals. **UNDER REVIEW**

Presentations

1. Featured Oral Presentation at Rapid Firing Session: The 10th Asia Pacific Heart Rhythm Society Scientific Session; Yokohama, JAPAN, September 2017

Abstract: Agbaedeng TA, Mahajan R, Munawar DA, Thanigaimani S, Elliott A, Thiyagarajah A, Twomey DJ, Khokhar KB, Lau DH, Sanders P. Systematic review and meta-analytic evidence for a relation between obesity and sudden cardiac death. *Journal of Arrhythmia* 2017

2. Oral Presentation at Rapid Firing Session: The 64th Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand, Perth, AUSTRALIA, August 2017

   Abstract: Agbaedeng TA, Mahajan R, Munawar DA, Elliott E, Twomey DJ, Khokhar KB, Lau DH, Sanders P. Obesity associates with increased risk of sudden cardiac death – a systematic review and meta-analysis. *Heart, Lung and Circulation* 2017;26(2):S186

3. Presented as a Poster: The European Society for Cardiology Congress; Barcelona, Spain, August 2017

   Abstract: Agbaedeng TA, Mahajan R, Munawar DA, Elliott A, Twomey DJ, Khokhar KB, Lau DH, Sanders P. Risk of sudden cardiac death in obesity – a systematic review and meta-analysis of emerging evidence. *European Heart Journal* 2017; 38(suplement_1)

4. Presented as a Poster: The Annual Scientific Conference of Heart Rhythm Society | Cardiac Electrophysiological Society; Chicago, USA, May 2017

   Abstract: Agbaedeng TA, Mahajan R, Munawar DA, Elliott A, Twomey D, Kumar S, Khokhar KB, Lau DH, Sanders P. Obesity and sudden cardiac death – a systematic review and meta-analysis. *Heart Rhythm* 2017;14(5):S205

Prizes

1. Annual Scientific Congress Travel Award, the European Heart Rhythm Association – Barcelona, 2018
2. National Scientific Meeting Travel Fellowship; the Cardiac Society of Australia and New Zealand – Perth, 2017
3. Adelaide Medical School Overseas Research Travel Award, the University of Adelaide – Chicago, 2017

Chapter 7:

Manuscript

Agbaedeng TA, Mahajan R, Thanigaimani S, Twomey DJ, Kuchel TR, Manavis J, Linz D, Lau DH, Mahajan R, Sanders P. Epicardial fat and fibro-fatty infiltration of the ventricle – implication for sudden cardiac death substrate in obesity. **UNDER REVIEW**

Presentations

1. Medical Staff Society Research Prize Presentation: The Annual Postgraduate Research Meeting of Royal Adelaide Hospital; Adelaide, AUSTRALIA, June 2018
   Abstract: Agbaedeng TA, Mahajan R, Thanigaimani S, Elliott A, Twomey DJ, Lau DH, Sanders P. Obesity drives fibro-fatty infiltration of ventricular myocardium – potential roles of desmoglein-2 and SMAD3-independent TGF-β pathways
2. Oral Presentation in New Discoveries in Genetics and Pathophysiology: Australian Society for Medical Research South Australia Annual Scientific Meeting, Adelaide, AUSTRALIA, November 2018

Abstract: Agbaedeng TA, Mahajan R, Thanigaimani S, Elliott A, Twomey DJ, Lau DH, Sanders P. Ventricular epicardial fat expansion and fibrofatty infiltrations – homing in on the arrhythmogenic mechanism of obesity

3. Featured Oral Presentation: The Frontiers in Cardiovascular Biology Annual Scientific Meeting; Vienna, AUSTRIA, April 2018

   Abstract: TA Agbaedeng, R Mahajan, S Thanigaimani, E Mclennan, DJ Twomey, DH Lau, P Sanders. Molecular characterisation of fibro-fatty infiltrations in the ventricular myocardium of obese sheep. *Cardiovascular Research* 2018;114(1):S11-12

4. Young Investigator Award Presentation: The European Heart Rhythm Association Congress; Barcelona, Spain, March 2018

   Abstract: TA Agbaedeng, R Mahajan, S Thanigaimani, A Elliott, E Mclennan, DH Lau, P Sanders. Ventricular structural remodelling in an ovine sheep model of sustained weight gain – potential role of desmoglein-2 in fibro-fatty replacement and arrhythmogenicity. *EP Europace* 2018;20(1):S110

5. Best Abstract Presentation: The 66th Australian Society for Medical Research Annual Scientific Meeting; Sydney, AUSTRALIA, November 2017

   Abstract: Agbaedeng TA, Mahajan R, Munawar DA, Thanigaimani S, Elliott A, Twomey DJ, Lau DH, Sanders P. Chronic weight gain results in ventricular intramyocardial fat cell infiltration and interstitial fibrosis in an ovine sheep model.

6. Featured Oral Presentation in Basic and Translational Science Session: The 10th Asia Pacific Heart Rhythm Society Scientific Session; Yokohama, JAPAN, September 2017

Abstract: Agbaedeng TA, Mahajan R, Munawar DA, Thanigaimani S, Elliott A, Thiyagarajah A, Khokhar KB, Akpoveso OOP, Twomey DJ, Lau DH, Sanders P. Abnormal biventricular remodelling in an experimental ovine sheep model of sustained obesity – implication for sudden cardiac death. *Journal of Arrhythmia* 2017

7. Ralph Reader Young Investigator Award Presentation: The 64th Annual Scientific Meeting of the Cardiac Society of Australia and New Zealand, Perth, AUSTRALIA, August 2017

   Abstract: Agbaedeng TA, Mahajan R, Munawar DA, Thanigaimani S, Elliott E, Twomey DJ, Khokhar KB, Akpoveso OP, Lau DH, Sanders P. Mechanisms of ventricular remodelling in a chronically obese ovine sheep model – implication for sudden cardiac death. *Heart, Lung and Circulation* 2017;26(2):S44

8. Featured Oral Presentation in Heart, Lung and Circulatory System: The Australian Society for Medical Research South Australian Annual Scientific Meeting; Adelaide, AUSTRALIA, June 2017

   Abstract: Agbaedeng TA, Mahajan R, Thanigaimani S, Munawar DA, Elliott AD, Lau DH, Sanders P. Obesity promotes pathological biventricular remodelling in an ovine sheep model – implication for sudden cardiac death

9. Young Investigator Award Presentation: The Annual Scientific Conference of Heart Rhythm Society | Cardiac Electrophysiological Society; Chicago, USA, May 2017

   Abstract: Agbaedeng TA, Mahajan R, Munawar DA, Thanigaimani S, Elliott A, Kumar S, Khokhar KB, Akpoveso OP, Twomey DJ, Lau DH, Sanders P. Ventricular fibrosis and epicardial fat cell infiltration of ventricular myocardium in an ovine sheep model of obesity – implication for sudden cardiac death. *Heart Rhythm* 2017;14(5):S514

Prizes

1. 1st prize, Young Investigator Award, the European Heart Rhythm Association Congress – Barcelona, 2018
2. Medical Staff Society Research Prize, Royal Adelaide Hospital – Adelaide, 2018
3. 3-Minutes Ph.D. Thesis Competition Finalist, South Australian Health and Medical Research Institute Annual Scientific Meeting – Adelaide, 2018
4. Ralph Reader Young Investigator Award Finalist, the Cardiac Society of Australia and New Zealand – Perth, 2017
5. Young Investigator Award Finalist, Heart Rhythm Society | Cardiac EPS – Chicago, 2017
6. Best Abstract Presentation, the Australian Society for Medical Research – Sydney, 2017
7. 3-Minutes Thesis Competition Finalist, the University of Adelaide – Adelaide, 2017
8. Annual Scientific Congress Travel Award, the European Heart Rhythm Association – Barcelona, 2018
9. National Scientific Conference Travel Award, the Australian Society for Medical Research – Sydney, 2017
10. National Scientific Meeting Travel Fellowship; the Cardiac Society of Australia and New Zealand – Perth, 2017
11. Adelaide Medical School Overseas Research Travel Award, the University of Adelaide – Chicago, 2017

Other Peer-reviewed Publications during Candidature

1. Munawar DA, Mahajan R, Agbaedeng TA, Elliott AD, Twomey DJ, Thiyagarajah A, Khokar K, Young GD, Roberts-Thomson K, Munawar M, Lau DH, Sanders P. Implication of ventricular pacing burden and atrial pacing therapies on the progression of atrial fibrillation – a systematic review and meta-analysis of randomized controlled trials. *Heart Rhythm* 2019;pii:S1547-5271(19)30142-0
2. Oehmke TB, Wu XY, Johnston JT, Gutiérrez C, Patel D, Moore EB, Lanzon E, Struett MM, Vergara AG, Agbaedeng TA, Sanganyado E, Park JJ, Halmhofer SJ, Nikolaou A, Mikhailova S, Winter KA, Gómez Luciano LB, Yang Z, Lang KM, Duong MT. Unique identities. *Science* 2019;364(6435):22-24

ABBREVIATIONS AND ACRONYMS

95% CI:	95 percent confidence interval;
ACROBAT-NRSI:	a Cochrane Risk of Bias Assessment Tool: for Non-Randomised Studies of Intervention;
AERP:	atrial effective refractory period;
AF:	atrial fibrillation;
Ang II:	angiotensin II;
APD:	action potential duration;
APD_{90}:	APD at 90% repolarisation;
ARVD/C:	arrhythmogenic right ventricular dysplasia/cardiomyopathy;
AT_1R:	angiotensin II receptor subtype 1;
BMI:	body mass index;
BP:	blood pressure;
CA:	catheter ablation;
CABG:	cardiopulmonary bypass grafting;
CFAE:	complex fractionated atrial electrograms;
CHI:	conduction heterogeneity index;
CICR:	calcium-induced calcium release;
Chi^2:	chi-squared;
CL:	cycle length;
CMR:	cardiac magnetic resonance imaging;
CPB:	cardiopulmonary bypass;
CS:	coronary sinus;

CT: computed tomography;

CV: conduction velocity;

CVD: cardiovascular disease;

DAD: delayed afterdepolarisation;

DEXA: dual-energy X-ray absorptiometry;

DF: dominant frequency;

DSG-2: desmoglein-2 protein;

EAT: epicardial adipose tissue;

EAD: early afterdepolarisation;

ECG: electrocardiogram;

ELISA: enzyme-linked immunosorbent assay;

ERP: effective refractory period;

ET-A: endothelin receptor subtype A;

EXP: explosive search;

Gal-3: galectin-3;

H&E: haematoxylin and eosin;

HR: hazard ratio;

I^2: I-squared statistic;

$I_{Ca\text{-}L}$: L-type calcium ionic current;

I_{K1}: inward rectifier potassium ionic current;

I_{KACh}: acetylcholine-activated potassium ionic current;

I_{Kr}: rapid component of the delayed rectifier potassium ionic current;

I_{Ks}: slow-conductance potassium ionic current;

I_{Kur}: ultra-rapid delayed rectifier potassium ionic current;

I_{K2P}: repolarising $K_{2P}3.1$ potassium ionic current;

$I_{Na\text{-}Late}$: late-sodium ionic current;

I_{to}: transient outward potassium ionic current;

IQR: interquartile range;

LA: left atrium/atrial;

LAA: left atrial appendage;

LAEF: left atrial ejection fraction;

LAT: local activation time;

LTCC: L-type calcium channel;

LVEF: left ventricular ejection fraction;

LVEDD: left ventricular end diastolic diameter;

LVESD: left ventricular end systolic diameter;

LVSD: left ventricular septal dimension;

MALDI: matrix-assisted laser desorption/ionization time-of-flight;

MCR: mineralocorticoid receptor;

MH: medical headword/headline;

MI: myocardial infarction;

MOOSE: meta-analysis of observational studies in epidemiology guideline;

MSI: mass spectral imaging;

NCX: sodium/calcium exchanger;

NOS: Newcastle-Ottawa scale;

NPAF: non-paroxysmal atrial fibrillation;

OPCAB: off-pump coronary artery bypass;

OR: odds ratio;

PAF: paroxysmal atrial fibrillation;

PAS: periodic acid Schiff;

PerAF: persistent atrial fibrillation;

POAF: post-operative atrial fibrillation;

PRISMA: preferred reporting items for systematic review and meta-analysis;

pSMAD3: phosphorylated SMAD 3 protein;

RA: right atrium/atrial;

RAA: right atrial appendage;

RAAS: renin-angiotensin-aldosterone system;

RAEF: right atrial ejection fraction;

RCT: randomized controlled trial;

RR: risk ratio;

RVEF: right ventricular ejection fraction;

RyR2: ryanodine receptor type 2;

SCA: sudden cardiac death;

SCD: sudden cardiac death;

SD: standard deviation;

SMAD3: sma and mothers against decapentaplegic homologue protein 3;

SMAD6: sma and mothers against decapentaplegic homologue protein 6;

SMD: standardised mean difference;

SR: sinus rhythm;

SYN: synonym;

TGF-β1: transforming growth factor-beta 1;

TTE: transthoracic echocardiography;

TβR1:	transforming growth factor-beta 1 receptor type 1;
TW:	title word;
VT/VF:	ventricular tachycardia/fibrillation

1. Chapter One

An Extended Literature Review

1.1 OBESITY – Scaling an epidemic

Obesity is a burgeoning condition associated with increased multi-morbid state in affected individuals.[1] More worryingly, the obesity epidemic is shown to lead to increased public health burden and associated social costs to communities.[1]

Data looking at trends in weight gain from the 80s through present times have consistently shown significant increment in the prevalence of obesity. In a recent Global Burden of Disease data, obesity was found among over 107 million children and 603 million adults, indicating a prevalence of 5.0% in children and 12.0% in adult population.[1] Conversely, the prevalence of underweight has tremendously decreased globally. According to data from the Non-Communicable Disease Risk Factor Collaborators, the global age-standardized prevalence of underweight decreased from 13.8% in 1975 to 8.8% in 2014, highlighting the overall shift in body mass index (BMI), which is defined as weight divided by height squared [$kg.m^{-2}$].[2]

Furthermore, future projections of obesity epidemic show increasing disease burden at alarming rates. Data from the Australian Department of Health puts yearly increase between 0.4% and 0.8%, meaning that by 2025, 83% of adult males and 75% of adult females will become either overweight or obese.[3] Similarly, over a third of children under the age of 20 years will become overweight or obese.[3] Taken together, approximately 7 million additional Australians will be impacted by 2025 as compared to 2005 figures.[3]

1.2 Obesity as a Cardiovascular Risk Determinant

Although it is known that obese individuals are at increased risk of developing adverse health conditions, its impact on cardiovascular health is recently being appreciated. Nonetheless,

obesity is shown to be associated with cardiovascular (CV) risk factors, such as diabetes[4, 5], hypertension[5], sleep apnoea[6], dyslipidaemia[5], and metabolic syndrome[7]. For example, sleep apnoea was reported to be prevalent in 18.9% patients with BMI <25 kg.m^{-2} rising to 82.85% in patients with BMI ≥40 kg.m^{-2}, and in the HypnoLaus study, both overweight (defined as BMI: 25 kg.m^{-2} to 29.9 kg.m^{-2}) and obesity (defined as BMI: ≥30 kg.m^{-2}) independently associated with 1.74- to 4-fold increased risk of sleep-disordered breathing in both men and women.[8] Interestingly, in the same study, adjustment for BMI resulted in marked reduction in the association of sleep apnoea with hypertension, type 2 diabetes and metabolic syndrome.[8]

When cardiac diseases are investigated, obesity is shown to significantly associate with increased risks, incidence, and disease prevalence. The risks of non-arrhythmogenic cardiac disease are greater in obese patients compared to normal weight counterparts. Even after correcting for advancing age and sex, the cumulative life-time risk of heart failure increases with increase in BMI classes, such that it doubles from 9 at BMI <25 kg.m^{-2} to 19 at BMI ≥30 kg.m^{-2}.[9] This situation is compounded further by evidence implicating obesity and overweight in the development of cardiometabolic multimorbid states in affected individuals. Using data from 16 prospective cohort studies across Europe and USA, Kivimäki et al[10] demonstrated that overweight increases the odds of cardiometabolic multimorbidity (characterized by type 2 diabetes, coronary artery disease and stroke) to twice those in healthy weight groups. In the same study, class I obesity (BMI: 30 to 34.9 kg.m^{-2}) resulted in 5-fold increased odds of developing multimorbid state, which increased to fifteenfold with severe obesity (classes II & III; BMI ≥35 kg.m^{-2}).[10]

1.3 Obesity and Atrial Fibrillation

1.3.1 Atrial fibrillation – Setting the scene

Atrial fibrillation (AF) is a rhythm disorder characterized by very rapid, chaotic electrical activities in the atria, culminating in accelerated and irregular ventricular activity, and loss of atrial mechanical function.[11] AF is the most common sustained arrhythmia present in the clinical setting, with a wide range of clinical presentations.[12-15]

AF is increasingly being recognised as a global clinical conundrum, and according to more recent epidemiological data, the occurrence of AF is predicted to surpass traditional cardiac conditions like ischaemic heart disease and heart failure.[12, 13] Importantly, patients with AF have increased risk of significant morbidity, with potentially deleterious outcomes.[13] The disease confers greater risk for stroke and systemic thromboembolism in affected patients[16, 17], such that a third of stroke events are caused by AF[18-20]. Interestingly, cardiogenic strokes due AF are more severe than other types of strokes; and a significant proportion of embolic strokes of unexplained source, termed "ESUS", are attributed to asymptomatic episodes of AF or subclinical AF.[18-21] Additionally, there are exaggerated risks of all-cause mortality and cardiovascular complications, such as CVD hospitalizations, and impairments of quality of life (QOL).[22, 23]

1.3.2 The Epidemiological Quandary of Atrial Fibrillation

AF is prevalent in 1% to 4% of the adult population in western countries, such as Australia, USA, and the European Economic Area, and is shown to rise above 13% by the 9th decade of life.[12, 13, 22] According to US population-based figures, the annual prevalence of AF in 2010

was estimated at 5.2 million from the 2.3 million reported in the ATRIA study by the end of the 20th century.[24] Additionally, in an age- and sex-adjusted meta-analysis, AF was found to be prevalent among 2.8% of adults.[12] Similarly, the global estimates show a growing pattern of AF prevalence. The 2010 Global Burden of Disease Study puts the age-adjusted AF prevalence rates at 596 per 100,000 males and 373 per 100,000 females, up from 569.5 in men and 359.9 in women in 1990, representing a 5% increase.[13] Consequently, AF is prevalent in staggering 33.5 million adult individuals, which, in fact, compares very well with global epidemics like cancers and HIV/AIDS, both, of which, are currently at 32 million and 36.7 million, respectively.[13]

A major contributor to the increased regional and global prevalence of AF is high incidence of new disease cases. In the Manitoba Follow-Up Study involving 3,983 participants, AF developed in 7.5% (299) during 154,131 person-years of observation.[25] In another study looking at community-based trends in AF incidence, Miyasaka et al[22] found 12.6% relative increase in AF incidence rate over 21 follow-up years, up from the age- and sex-adjusted incidence rate of 3.04 new AF cases per 1000 person-years at the end of first year to 3.68 by the end of the 21-year follow-up period. These are even higher when patients are stratified according to age, with up to 68.9 incident AF cases reported per 1000 persons per year by the 10th decade of life.[26]

The high lifetime risk of developing AF is another major cause for concern. Previous data reported sex-adjusted lifetime risk of AF ≥40 years of age at 25% and was shown to remain as high as 16% even after correcting for comorbidities, such as myocardial infarction (MI) or congestive heart failure (CHF).[27] More recently, modelling data by investigators from the Framingham cohort projected a 37% overall lifetime risk of the arrhythmia after 55 years.[28] Moreover, with the increasing risk factors like obesity, this is estimated to be even

higher. In fact, the presence of at least one risk factor was associated with 38.4% greater lifetime risk of AF compared to 23.4% in the absence of a risk factor.[28] Further, with the combination of rising ageing population, increasing incidence and high lifetime risks of the rhythm disorder, future projections show AF will be prevalent in a significant portion of the general population. This was very evident in the 2010 report by Chugh et al[13], entailing 5 million new cases of AF annually. Correspondingly, the US Census Bureau projected AF to be prevalent among 15.9 million by 2050, given that the increasing incidence is maintained.[13] Similarly, European-based models have projected 18 million patients to have AF, more than doubling the current rate at 8.8 million.[29] Most importantly, AF incidence and projection are, in most part, to be underestimated because of high rate of subclinical AF, which is rarely detected.

1.3.3 Risk Factors for AF

It is increasingly being accepted that AF is not a disease that occurs in isolation, or the so called “lone AF”. It is understood that multiple important clinical conditions, electrocardiographic and echocardiographic, and biomarkers predispose to episodes of AF, including increasing age, hypertension, heart failure, type II diabetes, valvular heart disease, obstructive sleep apnoea. Interestingly, data also show AF symptomatic burden increases with increasing number of these concomitant conditions in patients is associated with the chronicity of the rhythm disorder.

1.3.3.1 Hypertension

Hypertension (HTN) is a well-established cardiovascular risk factor. HTN is highly associated with increased incidence of AF. Indeed, HTN is very prevalent among individuals with AF – this was initially demonstrated in a Framingham Heart Disease study over a decade ago and is well corroborated by a large body of data till date, ranging from 50% to 90% of AF patients.[28, 30-32] Data show that HTN independently predicts new-onset AF, with future risk of AF increasing from 28% to 2.7-fold for every 10 mm Hg of systolic blood pressure (BP).[31] Independent long-term prediction of incident AF has also been noted for upper normal BP's (defined as systolic BP: 128-138 mm Hg and diastolic BP: ≥80 mm Hg), and at 50% and 79% increased risk, respectively.[33]

Hypertension is implicated in mechanisms driving AF. Both short- and long-term experimental models of hypertension have demonstrated increased inducibility and duration of atrial tachycardia and conduction abnormalities.[34-36] HTN promotes abnormal atrial cardiomyopathy, haemodynamic changes and release of neuro-humoral factors, all of which lead to formation of AF substrate. For example, the renin-angiotensin-aldosterone system, which is activated in HTN, is a predictor of cardiac arrhythmias.[37] Additionally, mechanical overload in hypertensive heart disease promotes chronic stretch causing structural remodelling of the atria, including myocardial fibrosis, left atrial (LA) hypertrophy, atrial inflammation and ion channel remodelling.[38] Interestingly, pharmacological control of BP in hypertension has shown promise in substrate regression and lower risk of new-onset AF.[39]

1.3.3.2 Obstructive Sleep Apnoea

Obstructive sleep apnoea (OSA) and central sleep apnoea (CSA) are the most common sleep-disordered breathing syndromes associated with worsening of health outcomes in adult individuals and shown to affect more than a quarter of these subjects globally.[6] The association of OSA with cardiovascular outcomes is well researched, with data supporting more than a triple prevalence rate of heart failure in OSA patients than in non-OSA patients.[40] Both OSA and CSA are shown to strongly predict AF, demonstrating 2- to 3-fold higher risk of developing future AF when compared to healthy cohorts.[41, 42] In untreated patients, OSA associates with higher recurrence of AF after DC cardioversion[43] and catheter ablation[44] and new-onset post-operative AF.[45] Moreover, patients in AF are more likely to present with OSA than do those in sinus rhythm.[46] Interestingly, treatment of OSA by continuous positive airway pressure is associated with reduction in AF recurrence rate and post-op AF after cardiac surgery, symptomatic burden and hospitalization.[47]

Obstructive sleep apnoea (OSA) is characterized by recurrent episodes of upper airway collapse during sleep caused by failure of the neuromuscular system to maintain airway patency. These repetitive cycles are what increase susceptibility to AF, wherein they lead to oxyhaemoglobin desaturation, sympathetic overdrive and vagal output, and excess generation of reactive oxygen species and intracardiac pressures.[48] Obstructive disordered breathing is associated with enlargement of intra-atrial area, intracardiac electromechanical delay, conduction delays, and development of low voltage.[49] OSA is also shown to promote AF maintenance, as typified by increased prevalence of right atrial rotors.[50]

1.3.3.3 Diabetes Mellitus

Diabetes mellitus is a well-known cardiovascular risk factor caused by autoimmune-mediated reduction of insulin in type 1 DM or insulin insensitivity in type 2 DM (T_2DM). Surmounting body of evidence shows that diabetes increases the risk of AF, and more than 2-fold incidence rate ratios of AF is seen in patients with T_2DM.[51, 52] In fact, hyperglycaemia and elevated glycated haemoglobin A_{1C} are positively correlated with increased mortality, recurrence of atrial tachyarrhythmias after catheter ablation[53], and AF pre- and post-CABG. Data on diabetes-mediated atrial remodelling are very limited. Streptozotocin-induced diabetic models show reduced connexin 43 phosphorylation and sinus node conduction delay.[54, 55] Diabetic rats also display induction of myocardial fibrosis with generation of atrial reactive oxygen species, such as advanced glycation end products (AGE).[56] Interestingly, development of fibrosis was halted in this model via blockade of AGE receptors, indicating potential of ROS in diabetic CMP-mediated atrial remodelling.[56]

1.3.3.4 Heart Failure

Heart failure (HF) and AF have interesting and complex relationships, often sharing similar risk factors and pathophysiological mechanisms. HF independently predicts future AF, increases PV reconnections after ablation[57] and thus predisposing to recurrent atrial arrhythmias[58], and AF progression[59]. AF is more prevalent in HF with preserved ejection fraction (HFpEF)[60, 61], but the prognostic severity is debatable[62]. The coexistence of both conditions is also noted and reported in more than 30% of patients by several prospective studies.[59, 63] Notably, incident HF is associated with worsening of outcomes in patients with AF, including higher risk of all-cause mortality[63], hospitalization, and bleeding. It seems that

the substrate for AF in HF is uniquely different from non-HF, often involving myocardial fibrosis and distinct remodelling of Ca^{2+}-handling but not increased refractoriness or conduction slowing.[64, 65]

1.3.3.5 Alcohol Excess

There has always been long suspicion of a link between excess alcohol intake and cardiac arrhythmias; however, this has not been thoroughly investigated.[66] In 1978, Ettinger et al[67] reported high prevalence of arrhythmias among patients with a history of heave alcohol consumption. Interestingly, positive correlation was seen between the months of high arrhythmia incidence and months of high occurrence of alcohol-related diseases, thus, leading the authors to coin the term "Holiday Heart."[67] During a long-term follow-up of 34,715 patients (>50 years), heavier drinking defined as >2 standard drinks per day was associated with 60% increased hazard of incident AF (HR: 1.60).[68] Conflicting evidence exists for the association between mild to moderate drinking and AF, with some studies to showing significant associations after multivariable adjustment[68], while others show no association[69]. At a mechanistic level, conduction disturbances (due to left atrial dilatation and reduction in conduction velocity)[70, 71] and impaired autonomic tone (reduced respiratory sinus arrhythmia after acute alcohol ingestion)[72] have been suggested as possible mediators of alcohol induced arrhythmogenesis. More recently, data from isolated human and murine atrial myocytes have indicated disturbance of calcium handling[73], which was mapped to stress signalling pathways[74].

1.3.3.6 Valvular Heart Disease

Changes in the heart valves are associated with abnormal remodelling of the myocardial walls and shown to predispose to arrhythmia.[75-77] Indeed, increased risk of AF is demonstrated in patients with valvular diseases, such as mitral stenosis and regurgitation, aortic stenosis and regurgitation[78], and tricuspid regurgitation[79]. AF was even suggested as a marker of more severe or long-standing mitral stenosis, and, in patients undergoing mitral balloon valvuloplasty, the presence of AF was associated with reduced procedural success, both short-term and long-term survivals, and event-free survival.[80] Moreover, the presence of AF has been shown to underlie comorbid valvular disease; coexistence of AF and mitral valve disease was recently associated with progression of tricuspid regurgitation and right-sided heart remodelling, which were both eliminated by surgical ablation of AF.[81]

1.3.3.7 Coronary Artery Disease

AF is a common finding reported in patients with coronary artery disease (CAD) and both conditions are known to coexist. In fact, up to 40% of AF patients are diagnosed with CAD[82, 83] and 74% have preclinical CAD[84]. Preclinical CAD is also reported to independently promote AF; thus, multivariate adjusted analysis shows independent association between coronary artery calcium score (CACS) and AF, with up to 3.2-fold greater hazards.[85] The presence of AF was associated with worse cardiovascular events in patients undergoing percutaneous intervention.[86]

1.3.3.8 Sinus Node Disease

The frequent occurrence of atrial arrhythmias in the patients with SND is long established, often owing to tachycardia-bradycardia syndrome or sick sinus syndrome.[87, 88] The incidence of AF is 10 times higher in SND patients than in the general population, which equates to 125 per 1000 person-years. In SND patients requiring pacemaker implantation, new AF diagnosis occurs in up to 68%, with permanent AF reaching 15% over the long term. Additionally, sick sinus syndrome is associated with 5.75- and 4.25-fold greater hazards of both prevalent and incident forms of AF.[89] Moreover, bradycardia due to SND is known to complicate AF management and is often the indication for pacemakers in 83% of SND patients.[89, 90]

There is evidence to suggest that this relation is also bidirectional, with AF predisposing to sinus node dysfunction and, subsequently, SND. Both of these conditions share similar pathophysiological substrates. In a study by Sanders et al[88], the authors demonstrated widespread atrial electro-structural abnormalities in SND patients, including left atrial enlargement, areas of low voltage and scarring, functional conduction delay, increased right atrial effective refractory period and loss of rate adaptation to ERP. Similarly, atrial tachyarrhythmias have been shown to cause reversible changes in the atrial tissue predisposing to SND.[91] In pre-clinical models, SND substrates due to AF have been mapped to: prolongation of sinoatrial node recovery time and reductions in intrinsic heart rates (induced by >2 weeks of rapid atrial pacing) in dogs[92]; calcium clock malfunction as typified by unresponsiveness to isoproterenol and repression of ryanodine type 2 receptors[93]. Taken together, these data suggest that AF is both a consequence and cause of SND. More importantly, sinus node dysfunction can create a self-perpetuating substrate for both SND and AF.

1.3.3.9 Chronic Stretch

The role of chronic stretch in abnormal atrial remodelling is well documented in reports from both pre-clinical and clinical studies over the last few decades. One example of chronic atrial stretch and volume overload in humans is the presence of an atrial septal defect (ASD) which is associated with atrial volume overload, increased atrial pressure and higher vulnerability to AF as compared to normal control subjects.[94, 95] Electro-anatomical mapping has demonstrated that ASD patients display atrial enlargement, an increase in low voltage areas suggestive of myocardial and electrical scar, an increase in electrogram fractionation and local conduction slowing.[94, 95] However, no change or an increase in ERP was observed.[94, 95] Further, mitral stenosis (MS) in patients leads to chronic atrial stretch with atrial enlargement and MS patients show a greater propensity for sustained AF without any changes in ERP.[96] Electro- anatomical mapping in MS patients revealed changes comparable to ASD patients.[96] Interestingly, in patients with MS, the atrial remodeling exacerbates greater physiological direction-dependent conduction characteristics compared to patients without chronic atrial stretch.[97] In another study, vulnerability and heterogeneity in ERP and conduction delay correlated with left atrial pressure in patients with MS.[98] Epicardial mapping of local electrograms and activation times in patients with mitral regurgitation and left atrial enlargement showed more extensive regions of conduction slowing during pacing and an increase in fractionated electrograms in the posterior left atrium.[99] Taken together, chronic stretch constitutes a major clinical correlate contributing to AF substrate formation.

1.3.4 Genetics of AF

Although AF is a very multifactorial in nature, it has become a common knowledge that individuals with the same number of risk factors do not have the same level of risk.[28, 30] In 2004, Fox et al[100], in Framingham cohort, demonstrated that AF status in parents was an independent predictor of future AF risk in offspring and was more exaggerated in the younger cohort; thus, indicating that the high variability in AF susceptibility might have some genetic component. Numerous other studies have also looked at the heritability of AF, often reporting similar findings.[101-105] It is interesting to note that the gene-attributable risk of AF tends to be higher in healthy patients with no known heart condition[101, 104] and younger individuals[100, 102, 103]. In a population-based national registry study involving over 4 million participants, Øyen et al[104] showed higher incidence rate ratios (IRR) of AF in patients who had familial AF in younger 1st-relatives; IRR of 5.42 patients ≤39 years old with familial AF in ≤39-year-old 1st-degree relative versus IRR 3.28 in patients ≤39 years with 1st-degree relative 40-59 years of age. More recently, there have also been heightened interests in gene variants modifying AF risk, AF substrate and circuits, and catheter ablation outcome, which are further explored below.

Evidence for a gene locus associated with AF was first reported by Brudaga et al[106] in 1997, where they mapped AF susceptibility gene to locus 10 by linkage analysis. Ever since, using linkage analysis, different authors have been able to identify gene variants that might precipitate heritable AF, such as: gain-of-function (GOF) mutation in potassium (K^+) channel α-subunit (*KCNQ1*)[107]; GOF mutation of sodium (Na^+) channel causing conduction disturbances and increased excitability (*SCN5A*)[108, 109]; natriuretic peptide precursor A associated with shortened APD[110]. Other reports have focused on single candidate genes to

see potential AF predisposing mutations. For example, A single-gene association study identified AF-associated variant in the slow component of the delayed rectifier K^+ current (I_{Ks}, R14C); with the authors concluding that, though by itself, R14C was insufficient to cause AF, it could precipitates higher risk of AF in the presence a "second hit" like hypertension.[111] Additionally, a heterozygous mutation (E375X) in the *KCNA5* gene encoding Kv1.5 (I_{Kur}) was identified in only AF patients but not in 540 unrelated controls.[112] The LOF mutation, which introduces a premature stop codon in the primary amino acid sequence and loss of the S4-S6 voltage sensor, pore-forming region, and C-terminus, associated with APD prolongation, early after-depolarisation, and increased vulnerability to triggered activity.[112] Interestingly, the authors were able to recapitulate the findings in a murine model.

As a legacy of the human genome project, it is now possible to search within a large expanse of the entire genome to find regions or loci that might associate with AF. Techniques like the genome-wide association studies (GWAS) have allowed the identification of several common variants or single-nucleotide polymorphisms (SNP) associated with AF, with the added advantage that they do not require multigenerational family cohorts as is the case in linkage analysis studies. Using an analysis of 316,515 SNPs in an Icelandic population (550 AF/AFlut patients and 4,476 patients), Gudbjartsson et al[113] identified rs2200733T and rs10033464T as AF-susceptibility variants on chromosome 4q25 and showed that for each additional copy an individual has, the risk of AF increases by 1.72 and 1.39, respectively. Notably, more than 30 AF-risk loci have been identified as of today, including at least 24 loci in people of European decent and at least 6 in Japanese populations.

Determining the mechanisms mediating these putative AF-risk conferring loci has remained a major difficult. To address this, Ritchie et al. looked at 33 AF patients and 17

controls and showed that the presence of two common variants in the locus of 4q25 (rs2200733 and rs10033464) determines clinical expressions of rare mutations in the ion channels and signalling molecules: SCN5A, NPPA, KCNQ1, KCNA5, and NKX2.5.[114] Similarly, increased inflammation[115], disruption of nucleocytoplasmic transport[116], non-pulmonary vein foci[117], and calcium handling- and extracellular matrix-receptor pathways[118] have been implicated in the causal link between genetic risk and AF. In summary, the discovery of genetic basis for atrial fibrillation has vast refined our understanding of the pathogenesis of the arrhythmia, and further characterisation of the associated loci will potentially improve the management of AF.

1.3.5 Mechanisms Driving AF – towards an understanding of the atrial substrate

AF is believed to require both an initiating spontaneous electrical activity and a permissive substrate for its development and progression.[11, 119, 120] The permissive atrial substrate consists in an enabling environment for the pathogenesis of AF and is characterised by the shortening of atrial refractoriness and re-entrant wavelength or by local conduction heterogeneities caused by disruption of electrical interconnections between muscle bundles; thus, AF substrate is created by both structural and electrophysiological atrial remodelling.[11, 120]

1.3.5.1 Structural Substrate for AF

The association of atrial structural remodelling with the pathogenesis of AF is well studied and has been reproduced in several animal models. Indeed, data from models of mitral-valve

regurgitation[121], right atrial pacing[122], and congestive heart failure (induced by 5 weeks of right ventricular pacing)[123] have shown that atrial changes promoting AF may range from gross tissue structural alterations (e.g. atrial fibrosis, myocyte hypertrophy and necrosis) to ultrastructural changes occurring at cellular levels of myocytes, such as accumulating glycogen and collagen. Abnormal morphological changes in the atria are also seen in clinical models. For example, Frustaci et al[124] demonstrated diverse changes in the atria, involving, hypertrophy, inflammatory infiltrates with myocyte necrosis, areas of patchy fibrosis and myofibrillolysis, in patients with lone AF.

Structural substrate for AF takes several weeks to months to develop. It is proposed that remodelling of atrial structure starts with ultrastructural changes progressing to more visible gross structural changes, thus stabilizing AF circuitry.[122] Of note, atrial fibrosis, myocyte hypertrophy, loss of contractile structures, and atrial inflammation are implicated as the most important factors causing structural remodelling.

1.3.5.1.1 The Atrial Fibrotic Story

Fibrosis is a key element in the formation and perpetuation of AF and is considered as the histological hallmark of structural remodelling.[64, 123] Induction of atrial fibrosis during formation of AF substrate is well reported in animal models. For example, 1 week of ventricular tachypacing-induced heart failure (tachycardiomyopathy) in dogs and resulted in extensive atrial interstitial fibrosis formation.[64] This has since been corroborated by work in transgenic mice overexpressing the pro-fibrotic transforming growth factor-beta 1 (TGF-β1)[125]; Zucker diabetic fatty rats[126]; experimental sleep apnoea in rats[127]; ventricular pacing-induced congestive heart failure[64, 123]; chronically instrumented sheep and rat models of

hypertension[35, 36]; and doxorubicin-induced non-ischaemic cardiomyopathy[128]. Involvement of atrial fibrosis in structural remodelling is also seen in human models of AF as well as risk factors, such as advanced age[129], mitral valve disease[121], dilated and hypertrophic cardiomyopathy[130].

Interstitial fibrosis contributes to atrial remodelling by altering muscle bundle architecture.[120, 131] Cardiac muscle cells are exquisitely arranged from end-to-end into contractile units of myofibres, which are further arranged into "bundles" of "fibres". Within each myofibre, myocytes are separated by thin layers of endomysial collagen tissue; in muscle bundles, myofibres are separated by perimysial fibrous tissue. Consequently, as fibrosis amount increases, myofibres may lose myocyte-to-myocyte connections arising from increased transverse separations. In a simulation work by Spach and Boineau, it was shown that such loss of side-to-side coupling of myocytes or myofibres can cause "non-uniform anisotropy", a discontinuous electrical conduction.[132] Interestingly, Lau et al[35] found significant negative correlation between atrial fibrosis with conduction velocity, and strong positive association with conduction heterogeneity index. So far, this has also been corroborated by several others demonstrating: significant correlation between increased endomysial fibrosis and complexity of fibrillatory conduction pathways and higher incidence of epicardial breakthrough; and higher atrial arrhythmia inducibility and longer AF durations with atrial fibrotic changes.[133, 134]

1.3.5.1.2 Myocyte hypertrophy – becoming too big is a bad idea!

Structural remodelling can also result from increase in myocyte size. Ausma et al[135] demonstrated up to 195% hypertrophy in atrial myocytes following chronic instrumentation

of goats by right atrial pacing. This is further demonstrated upon atrial dilatation (produced by right atriotomy and constriction of pulmonary artery)[136]; chronic atrial dilation (AF goats induced by 48-h burst pacing)[137]; in models of CHF; and during hypertension in chronically instrumented ovine and rats (produced by *O*ne *K*idney - *O*ne *C*lip nephrectomy model)[35]. Though the precise contribution of atrial cellular hypertrophy to substrate formation is not quite well defined, it is likely to be due to increased conduction pathway in the ensuing enlarged myocytes. To this end, Spach et al[138] analysed microscopic propagations in two-dimensional (2D) neonatal and adult cellular models, showing that increased cell size contributes to pronounced propagation delays during transverse propagation, and may be more important than patterns of gap junction distribution. It is also shown that conduction abnormalities maintaining AF circuits can be produced solely by myocyte hypertrophy without involvement of myocardial fibrosis.[137]

1.3.5.1.3 Gap junction remodelling – abnormal myocyte-myocyte connectivity

The heart muscle cells are lined by highly specialized, low-resistance channels, the *gap junctions*, for exchange of ions and small molecular weight (<1.5 kilodalton, kDa) molecules, such as adenosine triphosphate (ATP), cyclic-adenosine monophosphate (cAMP), inositol 1,3,5-trisphosphate (IP_3); and thus, allow for intercellular communication between neighbouring cardiomyocytes.[139] These junctions are formed by head-to-head joining of connexon hemichannels, made up of six four-transmembrane-spanning connexin (Cx) proteins, of which three are expressed in the atria (Cx40, 43 & 45).[140] Changes in quantitative expression of connexin proteins, distribution, location or composition have been implicated

in connexin-based atrial remodelling.[141-143] It should be noted that the evidence behind changes in the amount of Cx proteins has been rather conflicting, with some showing increased levels while others show reduced expression in AF.[141, 144]

Alteration in connexin proteins and ultimately gap junctions can cause abnormal electrical coupling of myocytes leading to conduction disturbances. Cx40 is particularly associated with conduction heterogeneity and is shown that partial or complete loss of Cx40 eliminates anisotropic conduction.[145] This suggests that, under conditions causing increased expression of Cx40, development of AF substrate will be favoured. Interestingly, Polontchouk et al showed significant association between Cx40 with chronic AF, such that 2.7-fold increased expression of Cx40 was noted in patients with chronic AF compared to SR, which was replicated with pacing-induced AF in rats.[141] Similarly, inhibition and ablation of Cx43 expression significantly decreases electrical coupling and increases inducibility of arrhythmias.[146] Cx43 can also promote remodelling by altering partner connexins and membrane currents.[146]

Further, Cx40 and Cx43 proteins and gap junction redistribution on myocytes may cause structural remodelling. Concomitant lateralisations of Cx40 and Cx43 have been observed in AF patients undergoing Maze procedure, in chronic AF and experimental AF model following rapid atrial pacing.[141, 144] The involvement of lateral gap junction remodelling in AF substrate is likely to be caused by reduced electrical coupling at intercalated disks and increased transverse conduction.[141]

1.3.5.1.4 Remodelling of ultrastructural architecture

Accumulating body of evidence shows that changes at subcellular levels, occurring early on before overt structural remodelling (1 to 3 weeks), may contribute to AF pathogenesis.[122] For example, Ausma et al[135] found marked subcellular changes, including loss of myofibrils, accumulation of glycogen granules, changes in mitochondrial shape and size, fragmentation of sarcoplasmic reticulum, and dispersion of nuclear chromatin, in over 92% of portion myocytes in goats with pacing induced AF. It is shown that mitochondria are smaller, greater in number (demonstrating elevated mitochondrial fission) and more elongated in chronic AF.[135] Mitochondrial dysfunction, typified by decreased adenosine diphosphate-stimulated respiration supported by palmitoyl-L-carnitine and mitochondrial permeability transition pore opening (indicative of increased Ca^{2+} sensitivity), was recently shown to predict post-operative AF.[147] Furthermore, there have been observations of ultrastructural changes involving amyloid deposition; dedifferentiation of myocytes by molecular switch to foetal phenotypes, such as re-expression of α-smooth muscle actin, abnormal distribution of titin and decrease in cardiotin; and loss of contractile fibres (myolysis) in AF conditions.[135, 148]

1.3.5.2 Electrophysiological remodelling

1.3.5.2.1 Abbreviation of action potential duration and refractoriness

Electrical remodelling leading to shortening of action potential duration (APD) and atrial effective refractory period (AERP) are well documented to increase AF vulnerability and stability.[149, 150] In goat and dog models of chronic AF, arrhythmia vulnerability was associated with decreased AERP (~50%; 150 ms to <80 ms), and heterogeneity in AERP reported as an independent predictor of AF.[149, 151] In clinical data, AF is associated with

reduction in rate adaptation to AERP and increased dispersion in refractoriness.[152] Similar to changes seen with refractoriness, APD is shown to be shorter in AF than in SR in both clinical and experimental models.[151] Changes in both AERP and APD occur very early on in the course of AF (first 3 days), much before the start of structural remodelling, and they are likely to precipitate decrease in atrial contractility seen during short AF episodes.[149]

Adaptation of atrial myocytes to rate changes in AF is proposed as a fundamental mechanism driving shortening of APD and AERP. This is reflected by modulations of ion channels which lead to pro-arrhythmic changes in membrane currents. In a model of heart failure induced by ventricular tachypacing for 4 months, there were significant shortening of atrial APD at 90% repolarization (APD_{90}, ~40%), APD_{50} (~60%), AERP; prolongation of the transient outward rectifier K^+ ionic current (I_{to}); and decreases in the ultra-rapid delayed (I_{Kur}), inward rectifier (I_{K1}) and slow-conductance (I_{Ks}) current densities; but, no change was found for the rapid component of the delayed rectifier current (I_{Kr}).[153] The involvement of late-sodium current ($I_{Na\text{-}Late}$) is also reported to impact atrial APD; it was shown to be markedly increased in castrated mice and was correlated with AF burden, rate and duration, which was ameliorated by inhibition of $I_{Na\text{-}L}$ with ranolazine, eleclazine or GS967.[154] Further, the repolarizing $K_{2P}3.1$ K^+ current (I_{K2P}) has been reported to modify atrial APD in chronic AF and pAF with advanced left ventricular dysfunction; inhibition of $K_{2P}3.1$ was reported to cause APD prolongation.[155]

1.3.5.2.2 Complex Fractionated Atrial Electrograms

Areas of complex fractionated atrial electrograms, known as CFAEs, have attracted much enthusiasm in the electrophysiology field as sites that may harbour substrates for perpetuating

fibrillatory circuits. Indeed, CFAEs have shown to represent areas of wave collisions, inhomogeneous tissue activations, slow conduction, and localised rotors.[156-159] In 1994, using right atrial (RA) high-density mapping in 25 patients with Wolff-Parkinson-White syndrome, Konings et al[160] made the seminal observation of fragmented wavefronts in the free wall of the RA. In some patients, activation was shown to be highly fragmented by arcs of intra-atrial conduction block producing multiple wavelets.[160]

These important findings have led to the development of the novel technique of CFAE ablation to treat PAF and persistent AF (PerAF). In 2004, Nademanee et al[156] demonstrated this in human AF (121 patients; 57 PAF and 64 permanent AF, PeAF) as a first-in-man modality. The authors defined CFAEs as ≥2 deflections on atrial electrograms (EGMs), and atrial EMGs of cycle lengths (CL) ≤120 ms. Using biatrial three-dimensional (3-D) electroanatomic mapping, the authors were able characterise AF by the regional distribution of AF, namely:

1. Type I (23 patients) – CFAEs are localised to one area and the rest of the atria have organised EGMs. Applied radiofrequency energy eliminated all CFAEs and terminated AF.
2. Type II (less organised, 43 patients) – CFAEs localised to two areas and ablation in both areas to terminate AF.
3. Type III – CFAEs localised in ≥3 areas (83% in interatrial septum) and was associated with multiple unsuccessful pharmacological cardioversions.

With the aid of CARTO 3D map, the investigators observed organisations of tachycardia EGMs following CFAE ablation, which ultimately led to elimination of AF.[156] Notably, the therapy was associated with high success rate at 1-year post-ablation follow up, demonstrating 91% freedom from both arrhythmia and long-term complications.[156] The

findings were further confirmed in subsequent publication by the investigators.[161] However, the hypes around CFAE ablation for AF treatment have not yielded much success in other trials.[158, 159, 162, 163]

Accurate characterisation if CFAEs remains a major challenge, an argument highlighted by proponents of CFAE-based ablation of AF. It is worth nothing that over eleven definitions of CFAEs have been adopted since research into EGM fractionations began about 50 years ago. For instance, the deflection threshold was defined as ≥3 deflections over the mapping area by Lee et al[164]; ≥2 deflections and/or continuous deflections of a prolonged activity perturbing the baseline, and waveform of cycle length ≤120 ms or shorter by Nademanee et al[156]; and just as CL of ≤120 ms or shorter than coronary sinus in the report by Oral et al[158]. The variation in software algorithms have also been noted. Over the decades, three software have been used in CFAE detection, including interval confidence level (ICL), complex fractionated electrogram (CFE) mean, and automated programs calculating CFAE percentage.

1.3.5.2.3 Dominant Frequencies

The concept of there been localised high-frequency sources of triggers for AF was put fort more than half a century ago. In 1931, using dog heart preparations, Brams and Katz[165] tested the concept of the presence of mother waves traveling in circus that would solely explain AF and ventricular fibrillations (VF). The authors made an important observation that fibrillatory waves continue even after functional and/or anatomical separation the atrial chambers, thus highlighting role of local activity in AF.[165] Almost 60 years later, Schuessler and colleagues[166] showed, in canine RA preparation, multiple re-entrant circuits that tended to

convert to small, relatively stable and high-frequency dominant activation with increasing concentration of acetylcholine treatment. Two subsequent experimental reports further confirmed these finding by demonstration of: focus of very short AF CL in the posterior LA wall of halothane-anaesthetised dog, which were successfully cryoablated[167]; and successful ablation (62% to 67%) of dominant focus with short CL in the Bachmann's bundle in sterile pericarditis canine model[168]. Clinical evidence for DFs was initially reported in nine patients with PAF, wherein the authors observed dominant focal and rapidly firing activity source that associated with AF and was successfully eliminated with RF ablation.

DF substrate tends to exhibit heterogeneities depending on the type of AF (PAF or PerAF). In an acute canine model, Sih et al[169] demonstrated shorter mean AFCL in the LA than RA, with DF originating from the posterior and medial portion of the LA. In the chronic model, the LA-RA AFCL gradient exhibited greater severity and 25% increased disorganisation of LA activation, but no change in the RA. The existence of DF gradients has been confirmed in multiple preclinical investigations[170, 171]. Moreover, ablation of sites exhibiting left-to-right gradients is associated high long-term ablation success rate.[172] Furthermore, a single DF was identified in 94% acute AF dogs and 57% in PerAF dogs[173]. Also, in human, high DF sites were reported to be higher in the LA-PV junction than in coronary sinus (intermediate) and posterior RA (lowest) in PAF, but no difference was observed in PerAF[174]. Using spectral analysis and frequency mapping modalities, Sanders et al[157] showed that, in patients with PAF, high DF sites are more localised around the PV, whereas, in PerAF patients, DFs are more widely distributed. There reports showing that combination of DF- and CFAE-based CA ablations results in high success rates, thus indicating that DF mapping may revolutionise substrate-based AF ablation.[157] Moreover, a recent report using the automated CARTOFINDER system and PentaRay recordings, DF and

CFAE demonstrated comparable correlations with low voltage zones in predicting focal and rotational AF drivers.[175]

1.3.5.2.4 Conduction Slowing and Conduction Heterogeneity

Abnormalities of atrial conduction are known to be important for electrical substrate formation. One of the first demonstration for abnormal conduction patterns comes from periprocedural induction of AF in Wolff-Parkinson-White (WPW) syndrome patients undergoing surgery.[160] Whereas, homogenous conduction is usually observed in the right atrium of WPW patients in sinus rhythm, AF was associated with complex conduction along plenty arcs of block.[160] In patients with PeAF, rapid repetitive patterns of conduction were reported, implying that increasing heterogeneity in conduction can serve as self-perpetuating substrate for more chronic forms of AF. Correspondingly, Allesie et al[176] performed epicardial mapping in 24 patients undergoing cardiac surgery to characterise fibrillatory conductions. The authors found more significantly impaired conduction in PerAF compared to acute AF, with the former having 6-fold higher lines of blocks than the latter.[176] They also found electrical dissociation between neighbouring muscle fibres, which were more severe in the PVs than in the free wall of the RA.[176] In a parallel report by the same authors, they demonstrated that these dissociated fibrillatory wavelets are epicardial breakthrough waves. The authors concluded that epi-endocardial dissociation and longitudinal dissociation of atrial muscle fibres constitute the second most important substrate for PerAF[177].

In preclinical models, conduction disturbances have also been demonstrated. Earlier reported showed both increased and reduced conduction velocities (CVs) following tachycardia induced by rapid atrial pacing.[167, 178-180] However, in these studies, there was no

change in CV heterogeneity (CHI). More recently, impaired conduction has been demonstrated in: rat model of hypertension and ageing[36]; hypertension in chronically instrumented ovine model[35]; ovine models of overweight and short- and long-term obesity[181, 182]; goats with short- and long-term AF[134]. Notably, Verheule et al[134] made the important finding that presence of endomysial fibrosis, characterised by separation between muscles within bundles, in occur more frequently in chronic AF. intriguingly, the goats with chronic AF had slower wavefront expansion and more anisotropy during high-resolution optical mapping.[134]

1.3.5.2.5 Sinus Node Dysfunction

Sinoatrial (SA) node disease (SND) and AF are known to have bidirectional relation. Numerous studies show that AF predispose to SA dysfunction and, subsequently, SND. Atrial tachyarrhythmias promote left atrial enlargement, areas of low voltage and scarring, functional conduction delay, increased right atrial effective refractory period and loss of rate adaptation to ERP. Similarly, atrial tachyarrhythmias have been shown to cause reversible changes in the atrial tissue predisposing to SND.[91] In pre-clinical models, SND substrates due to AF have been mapped to: prolongation of sinoatrial node recovery time and reductions in intrinsic heart rates (induced by >2 weeks of rapid atrial pacing) in dogs[92]; calcium clock malfunction as typified by unresponsiveness to isoproterenol and repression of ryanodine type 2 receptors[93]. Tachycardia-induced SA node remodelling does not occur very quickly. Manios et al[183] observed the recovery of AF induced remodelling occurred over 24 hours. In a study of patients with paroxysmal and chronic atrial flutter, Sparks et al[91] noted that prolongation of SA node recovery time reversed in 5 min for paroxysmal flutter. However,

even after termination of flutter, reversal of SA node remodelling did not occur till after 3 weeks.[91] Taken together, these data suggest that AF is both a consequence and cause of SND. More importantly, sinus node dysfunction can create a self-perpetuating substrate for both SND and AF.

1.3.5.2.6 Abnormal calcium-handling and sensing

As a conceptual framework, excitation-contraction coupling is initiated by the entry of Ca^{2+} into the cells via the L-type $Ca_v1.2$ channel during phase 2 of action potential, leading to increased subsarcolemmal $[Ca^{2+}]_i$ that activates and causes opening of type 2 ryanodine receptor (RyR2) that allows for the surge in systolic Ca^{2+} levels (**Figure 1**).[184, 185] Diastolic Ca^{2+} level is maintained via the activities of the ATP-dependent sarco/endoplasmic reticulum Ca^{2+}-ATPase type-2a (SERCA2a)[186], Na^+/ Ca^{2+}-exchanger (NCX)[187], plasmalemmal Ca^{2+}-ATPase (PMCA)[188-190]. Interestingly, $[Ca^{2+}]_i$ is highly regulated, such that dysfunction in any of its regulating arm can lead to a pro-arrhythmic state. Increased $[Ca^{2+}]_i$ is associated with increased activation of NCX resulting in influx of Na^+ (producing transient inward current), which leads to afterdepolarisations (known contributors to APD alternans); ion channel remodelling; contractile dysfunction. Yeh et al[191] reported, in congestive heart failure induced by 2 weeks of ventricular tachypacing in dogs, significant increases in atrial diastolic $[Ca^{2+}]_i$, $[Ca^{2+}]_i$ transient amplitude, and sarcoplasmic reticulum (SR) Ca^{2+} overload. The authors correlated these events with spontaneous Ca^{2+} transient events and triggered activity, which were suppressed by RyR2 or NCX blockade.[191]

Abnormal Ca^{2+} handling can occur as dysfunctions in L-type Ca^{2+} Channel ($Ca_v1.2$, LTCC), RyR2, SERCA2a or calsequestrin-2 (CASQ2). RyR2 controls Ca^{2+}-induced Ca^{2+}

release (CICR) from the SR, and RyR2 remodelling is implicated as the most important Ca^{2+}-handling factor in AF pathogenesis.[191-193] In a canine model of CHF, Kubalova et al[194] detected increased sensitivity of RyR2 to luminal $[Ca^{2+}]_i$ and reduced receptor content, which correlated with increased Ca^{2+} sparks. This increased open probability of RyR2 has been attributed to hyperphosphorylation of the receptor by protein kinase A (PKA) or Ca^{2+}-Calmodulin-dependent protein kinase II (CaMKII) in human atrial myocytes.[193, 195] Regulation of important interacting partners of RyR2 may impact CICR; for example, arrhythmia-promoting mutation has been noted in junctophilin-2 (JPH2), the protein responsible for maintaining the junctional membrane complex for ECC; and increased open probability of RyR2 in transgenic mice caused by reduced expression of protein phosphatase 1 (PP1) is also seen.[196] Further, luminal Ca^{2+} $[Ca^{2+}]_i$ is tightly regulated by SERCA2a, CASQ2 and phospholamban (PLB), such that reduction in SERCA2a is correlated with AF persistence.[197] Ca^{2+}-handling abnormality involving LTCC is seen as decreased peak $I_{Ca\text{-}L}$ noted in an ageing model of Welsh Mountain sheep[198]; significantly reduced mRNA expression levels in AF patients[199]; or decreased α1C subunit with consequently increased Ca^{2+} transients in aged spontaneously hypertensive rats[200].

1.3.5.3 AF triggers

Events originating as ectopic or focal discharges and re-entrant circuits are thought to serve as initiating mechanisms for AF.[11, 119, 120] These can be organized as “hierarchical” propagations, wherein the AF drivers are from localized sources, or in an “anarchical” fashion, involving firing from multiple non-localised sources.[11]

Ectopic activity is promoted by abnormality in atrial cellular electrophysiology. **Figure 2** shows an illustration of the normal AP generation, involving 5 phases, AP upstroke by the depolarizing current of Na^+ currents (I_{Na}, phase 0), the initial extrusion of Na^+ (phase 1), plateau phase due to both opening of LTCC and CICR by RyR2 activation (phase 2), early repolarization by outward currents (phase 3) and late repolarization by I_{KACh} and I_{K1} (phase 4). Abnormal or enhanced automaticity can be caused by the presence of AF-promoting conditions that cause membrane potentials to become more positive, thus allowing for spontaneous AP generation.[201] It should be noted that enhanced automaticity is not easily demonstrated as a mechanism of AF.

Afterdepolarisations, including early and delayed afterdepolarisations (EADs & DADs), producing triggered activity are thought to be the main drivers of focal arrhythmias. EADs are due to mechanisms causing prolongation of AP and are responsible for tachyarrhythmias associated with long QT syndrome.[202] These mechanisms may occur as potentiation of late non-activating Na^+ current, $I_{Na,L}$, or reduction in repolarizing K^+ currents; the ensuing APD prolongation is able to allow recovery of LTCC from Ca^{2+}-mediated inactivation leading to Ca^{2+} influx and AP in phase 2 or 3 (**Figure 2**).[202, 203] DADs are caused by mainly Ca^{2+}-handling abnormalities that allow for spontaneous SR Ca^{2+} leak and diastolic SR Ca^{2+} release events.[195] Diastolic SR Ca^{2+} release leads to cytoplasmic Ca^{2+} overload that activates the electrogenic NCX, which is able to exchange Ca^{2+} for influx of depolarizing I_{Na} (1:3 stoichiometry) and trigger AP.[204]

1.3.5.4 Mechanisms Sustaining AF

Re-entrant mechanisms are currently understood as the main drivers of persistent forms of AF. Both functional and structural remodelling contributes to the formation of re-entrant circuitry. Several theories have been postulated over the years to explain the functional determinants of re-entry, such as:

1. The circus movement,
2. Leading circle,
3. Spiral circus, and
4. Multiple wavelets hypotheses (**Figure 3**).[11, 120, 205-207]

1.3.5.4.1 Anatomical Re-entry

In circus movement re-entry, re-entry occurs in the presence of a unidirectional block wherein an activation travels along the anatomical block or pathway to re-excite a previously excited region.[208-210] It follows that this mechanism requires full recovery of a previously excited tissue before the activation arrives.[211]

1.3.5.4.2 Functional Re-entry

The leading-circle concept posits that re-entry is through a central region constantly being activated by rotating waves. It was first postulated by Garrey[207] in 1924 and experimentally demonstrated by Allessie et al[208] in 1973 in isolated rabbit left atrial tissue as requiring no anatomical obstacle. The dimension of this re-entrant circuit is equivalent to wavelength (defined as product of refractory period and conduction velocity) and adapts to the smallest-

sized loop to maintain re-entry. The short wavelength of the leading-circle means the chance of spontaneous termination is low, thus higher likelihood of AF sustenance.

As a limitation of the leading-circle model, it fails to account for some observations made, such as; the anti-arrhythmic effects of Na^+-blocking agents which reduce both conduction velocity and wavelength[212]; and the unchanged wavelength seen in some experimental settings and in some AF patients[213]. The spiral-wave model was developed to settle this and is initiated when an activation wavefront encounters anatomical obstacle and circulates or rotates around it (also termed "Rotor theory").[11, 120, 205] This is best represented by the intricate relationship between the source and sink of electrical depolarization, with source being a recently activated region and sink, the region beyond that is still refractory.[11, 205] The wavefront curvature increases along the rotating spiral wave from regions with less source-sink mismatch to regions with the critical mismatch, often termed the rotor core. Interestingly, rotors have both been observed experimentally and in humans. In humans, rotor ablation has been associated with high success rates and, as seen in critical examples, leads to 80% to 95% freedom from AF.[214, 215]

1.3.5.5 Molecular Mechanism of AF

While the formation of AF substrate and eventual perpetuation of AF circuits can be understood as involving gross structural, electrical and functionals changes, induction of cascades of abnormal signalling are also implicated. Data from animal and clinical studies have implicated signals from, but not limited to oxidative stress pathway; inflammatory pathway; autophagy; mitochondrial dysenergetics; Ca^{2+}-modulating pathways; fibrotic signalling; and microRNA (miR) pathway.

1.3.5.5.1 Oxidative Stress

Oxidative stress, characterized by excess reactive oxygen species (ROS) and reactive nitrogen species (RNS), is shown to be higher in patients with AF[216, 217], recurrent arrhythmia after catheter ablation[218], and disease progression and post-operative form of AF[219, 220]. In mice, chronic cardiac overexpression of Rac1 GPase, an activator of nicotinamide adenine dinucleotide phosphate hydrogenase (NADPH) oxidase (NOX)-2, results in spontaneous development of AF, highlighting role of oxidative stress in atrial substrate formation.[221, 222] Myocardial ROS production tends to show differential temporal and spatial alterations. This was demonstrated by 1 weeks of AF in a porcine model; here, both Rac1 levels and NADPH oxidase activity were increased in left atrial tissue but no observable difference was seen in the right atrium compared to sinus rhythm.[223] In the same study, short-term AF was associated with higher superoxide ($^{\cdot}O_2^-$) production, with similar results seen in post-operative AF.[223] In contrast, long-term AF in goats[224] and permanent AF in humans[225] demonstrated a shift in O_2^- production from NOX2-NADPH oxidase to uncoupled nitric oxide synthase (NOS) and mitochondrial complexes.

Mechanistically, the available body of data suggest oxidative stress contribute to AF substrate via oxidation and nitrosylation of targets. Indeed, oxidation of a regulatory methionine unit in the Ca^{2+}/Calmodulin-dependent kinase II (CaMKII) by increased ROS levels has been reported.[226] Oxidized CaMKII shows increased activity, with increased excitatory phosphorylation of RyR2 on serine residue 2814 leading to greater Ca^{2+} sparks, impaired Ca^{2+} handling and greater susceptibility to AF in atrial myocytes.[227, 228] RyR2 is also a target of oxidation during states of oxidative stress and, in atrial myocytes, oxidized

RyR2 is significantly increased during chronic AF, with greater open probability.[229] Accordingly, genetic ablation of mitochondrial ROS production in a transgenic mouse model of leaky RyR2 was associated with reduced RyR2 oxidation and diastolic SR Ca^{2+} leak and AF induction.[229] It is also interesting to note that ROS/RNS can act as signalling molecules and activates abnormal myocardial signalling. This is seen with: increase in expression of phospho-c-Jun N-terminal kinase 1, MAPK p38 and MMPs following H_2O_2 stimulation of atrial fibroblasts[230]; and reduction in NF-κB, cell apoptosis, fibrosis and hypertrophy after advanced glycation end-products' blockade with myricitrin in mice with diabetic cardiomyopathy[231].

1.3.5.5.2 Abnormal Autophagic Events

The autophagy pathway is a newly implicated molecular pathway in cardiac conditions. Physiologically, autophagy serves to protect cells and maintain cellular homeostatic control by clearing abnormal cellular structures (abnormal or misfolded proteins, dying organelles).[232] Disease state ensues when this pathway is inhibited or hyperactivated. Several aspects of autophagy have been observed in models of ischaemia, ischaemia/reperfusion injury, mitral and tricuspid regurgitation, and hypertrophy, with both protective role and detrimental effects noted.[233-236] Autophagy has been associated with reduced expression of Cx43 and ionic currents, indicating a likely remodelling of electrical coupling of myocytes.[237, 238] In humans, electron micrograph of RAA samples of patients who went on to develop POAF following CABG demonstrated marked autophagosome vesicles but no statistically significant difference in fibrosis or inflammation.[239] Greater processing of the microtubule-associated protein 1B-light chain 3(LC3B)-I to LCB3II, indicating formation of

double-membrane autophagosome cellular substructures, has been reported in human AF as well as pacing-induced arrhythmia in animal models.[237, 240] Moreover, increased phosphorylation of AMP-induced protein kinase (AMPK) has been reported in AF, highlighting activation of the autophagic process consequent to tachycardia.[241]

1.3.5.5.3 Inflammatory Signalling

The infiltration of inflammatory cells and cytokines that mediate inflammatory response in myocardial tissue is associated with AF. Both local and systemic inflammation independently predict incident AF and recurrence after catheter ablation, cardioversion, and cardiac bypass. Inflammation is also implicated in both electrical and structural remodelling.[242] Samir et al[243], in a seminal paper, showed that increased fibrotic remodelling, reduced contractile function and abnormal Ca^{2+} transients in a transgenic mice model with atrial overexpression of tumour necrosis factor-alpha (TNF-α). In the same study, programmed stimulation resulted in induction of re-entrant atrial arrhythmias in isolated perfused hearts from TNF-α animals compared to controls.[243] Myeloperoxidase (MPO) is increased in patients with AF[244] and, in animal studies, ablation of MPO led to reduction of MMP-2 and -9 and fibrosis in mice treated with angiotensin II, effects that were rescued upon restoration of MPO[245]. Activation of the NLRP3 inflammasome has also been implicated in the formation of atrial substrate, involving myolysis, cardiomyocyte apoptosis, fibrosis, abnormal Ca^{2+}-handling, shortening of refractoriness, and increased AF inducibility.[246, 247]

1.3.5.5.4 MicroRNA-mediated Atrial Remodelling

MicroRNAs (miRNAs) and short non-coding RNA sequences are emerging as very useful biomarkers of AF. Circulating levels of miRs have been associated with greater risk of AF presence[248], incidence[248], progression, and recurrence post-catheter ablation[249-251]. In a cohort of 34 patients undergoing on-pump cardiac bypass grafting, higher pre-operative levels of miR-483-5p predicted greater incidence of new-onset post-operative AF[252]. There are even observations of altered expressions of miRs in the atrial tissues of AF patients versus AF-free individuals, thus underscoring a role for miR in AF substrate formation[253, 254]. This was recently demonstrated in an experimental model, wherein the clustering of miR-23b-3p and miR-27b-3p induced atrial fibrosis via activation of TGF-β receptor 3 (TGF-βR3)/SMAD3 signalling pathway. Additionally, in heart failure after MI model, treatment with miR-21 blocking agent (KD21) significantly reduced atrial fibrosis and AF induction.[255] The repression of fibrosis was shown to be mediated by sprouty-1, an inhibitor of the pro-fibrotic ERK pathway, and miR-21 levels were negatively correlated with sprouty-1 levels in humans with valvular AF.[255] Furthermore, miRs have been associated with induction of electrical remodelling[256, 257] and conduction abnormalities[258, 259] in preclinical models. Overexpressions of miR-130a and miR-206 have been shown to cause downregulation of connexin-43 with subsequent abnormal PR intervals, induction of cardiac arrhythmias, and shortening of lifespan.[258]

1.3.6 AF Association with Adiposity

The link between obesity and overweight and risk of AF was not evaluated until the turn of the 21st Century. There is strong rationale to suggest that obesity may precipitate AF

development. Obesity is associated echocardiographic markers of atrial dysfunction and shown to associate with atrial enlargement.[260] Both AF and obesity are parallel burgeoning global conditions, trends that persist even in the wake of declining incidence of traditional risk factors. Moreover, obesity can co-segregate with and worsen cardiometabolic risk factors that are known to promote the development of arrhythmias, including HTN, T2D, and heart failure.[1, 4, 7]

In a seminal paper by Wang et al[261] in 2004, the authors demonstrated increasing age-adjusted incidence rates for AF across BMI subgroupings (9.7 per 1000 person-years in BMI <25 kg.m^{-2}, 10.7 in BMI 25 to 29.9 kg.m^{-2}, and 14.3 in BMI ≥ 30 kg.m^{-2}), and after adjustment made for CV risk factors and interim MI or CHF, 1-unit increase in BMI was associated with 4% greater risk of AF. Also, in the same study, obesity predicted a 52% increased risk of AF in males and 46% in females independently of traditional risk factors, including MI and CHF, highlighting obesity as important modifiable risk for AF.[261] To further test the strength of this association, Wong et al[262] conducted a meta-analysis by pooling multivariable adjusted odds ratios from 51 cohort and case-control studies. Quite intriguingly, the authors found 19% to 29% elevated risk of incident AF for every 5-unit increase in BMI.[262] Additionally, BMI was also associated with recurrence of AF after catheter ablation and development of *de novo* AF following coronary artery surgery.[262]

These findings beg the question that obesity might actually underlie pathophysiological changes promoting AF. Intriguingly, visceral adiposity has been shown to correlate with systemic pro-inflammatory state, worsening of atrial haemodynamics, markers of atrial structural remodelling, and neurohumoral system including autonomic tone dysregulation, all of which are strongly linked to promoting atrial arrhythmias.[260] Similarly, there is experimental data suggesting that obesity leads to global biatrial endocardial

remodeling which is characterized by LA enlargement, fractionated electrograms, conduction abnormalities, diffuse atrial fibrosis.[182]

1.3.7 Arrhythmogenicity of Epicardial Fat

There is growing evidence to suggest that the increased risk of AF in obesity may be driven by expansion of epicardial adipose tissue (EAT), which is a sequela of increased adiposity seen during weight gain.[263] Indeed, there has been an explosion of interests in this ectopic adipose depot, no less due to its strategic location. Important considerations for arrhythmogenic roles of epicardial fat are discussed below.

1.3.7.1 Clinical Link between EAT and AF

The clinical relation between epicardial fat and AF was first reported by three independent cohorts. In the Framingham Heart Study Offspring and Third Generation Cohorts, analysis of the pericardial fat by multi-slice computed tomography (MDCT) in 3217 individuals showed significant association with prevalent AF.[264] This persisted even after correcting for AF risk factors, BMI and visceral fat. Interestingly, neither intrathoracic nor visceral abdominal fat associated with AF presence.[264] In another study, Al Chekakie et al[265] demonstrated lager volumes of total epicardial fat (quantified by CT) in patients with prevalent AF, which further associated with greater persistence of the arrhythmia. Additionally, Batal et al[266] evaluated the hypothesis whether a more local ectopic fat may be impact AF risk. Using 169 consecutive patients undergoing CT angiograms, they found that increased peri-atrial epicardial fat thickness at the posterior left atrium conferred more than 5-fold greater odds for AF.[266] The associations have so far been replicated in other cohorts and by other imaging

modalities. For instance, Wong et al[263] showed by cardiac magnetic resonance infrared scans that pericardial fat volumes, but not markers of general adiposity such as body mass index or body surface area, were independent predictors of prevalent AF.

1.3.7.2 Characteristic Behaviour of EAT

The EAT, like commonly found in other adipose tissue, contains not only adipocytes but non-fat mesenchymal cells, including the resident macrophages, pre-adipocytes, lymphocytes, adipose tissue-derived stem cells. The expansion of EAT can be understood as both involving the proliferation and the differentiation of pre-adipocytes. Whether both are equally responsible for an expanding epicardial fat, or one is the prevailing mechanism of EAT accumulation is yet to be determined.

The activity of EAT is also poorly investigated, including the profile of its secretome, their perceived contribution to LA remodeling and paracrine regulations. Recently, one study indicated that human EAT has specific regional and anatomic transcriptomic signature, depending on whether it is peri-atrial (PA-EAT), ventricular (PV-EAT), or coronary (PCA).[267] In this study, transcriptomic analyses of EAT and thoracic subcutaneous fat (SAT) of 41 patients matched for AF, CAD, and CV risk factors showed up to 2,123 (at false discovery rate, FDR, 5) and 2,728 (FDR of 10) genes significantly up-regulated in total EAT as compared to SAT, with 400 of these genes commonly shared across the EAT stores.[267] Interestingly, the commonly shared genes were members of the gene families controlling extracellular matrix remodeling, inflammation, infection, and thrombosis.[267] There was a disproportionate expression of these genes in the various depots, with PV EAT having overexpression of uncoupling protein 1 (UCP-1); PCA EAT having overexpression of

proliferation, *O-N glycan* biosynthesis, sphingolipid metabolism specific genes; and PA EAT specific for myocyte contractile and calcium signalling genes.[267] More intriguingly, similar number of genes was down-regulated in EAT using SAT as a reference. While these results implicate differential gene expression of EAT depots, they raise important questions regarding what happens during AF substrate formation. For example, what would the behaviour of the secretome of EAT be during obesity or tachycardia/AF? UCP-1-expressing adipocytes are shown to be protective, with the characteristic non-shivering thermogenesis. AF patients are reported to have reduced expression of UCP-1 mRNA in EAT and UCP-1 is negatively correlated with LA dilatation, with the beige adipocyte phenotype (reduced during EAT expansion) shown to be an independent predictor of AF.[268] Most importantly, if these pathways are remodelled in obesity or AF, are they due to EAT adipocytes or the stromal components of the adipose tissue?

Some of these questions were recently investigated by Chilukoti et al[269] in a human model of AF and an experimental model of rapid atrial pacing (RAP). After a 7-hour long pacing of pigs at 600-ms, RAP was shown to alter the expression of 66 genes controlling adipogenesis and adipocyte differentiation as shown by their elevated mRNA levels, which were confirmed upon 7 days of *in vitro* differentiation of 3T3-L1 fat cell lines after 7 days.[269] On the other hand, only a fraction of these genes, such as the metabolism-regulating genes *RETN, IGF-1, HK-2, PYGM, LOX, and NR4A3*, were differentially overexpressed in RA tissue samples of patients with AF as compared to controls, indicating more metabolic adaptation during AF.[269] Further characterisation showed significant increase in expression of *RETN* in EAT of AF patients compared to controls, suggesting an induction of inflammatory program in fat pad.[269] The results also showed that AF might activate a transcriptional maladaptation in EAT that favours tissue expandability and lipid accumulation as indicated

by increased expressions of *NR4A3* in atrial tissue and *ANGPTL4* in EAT of AF patients.[269] Noteworthy, these observations are limited by lack of data looking at obese vs non-obese models.

1.3.7.3 Inflammation and Epicardial Fat

Acet et al[270] evaluated the neutrophil-lymphocyte ratio (NLR), a highly validated marker of systemic inflammatory response, in AF populations and showed a significant correlation with epicardial fat thickness ($r = 0.66$; $p<0.001$), which was independent of other co-variates ($p<0.001$). In another study, the inflammatory activity of epicardial fat using F-18-fluorodeoxyglucose (FDG)-PET/CT in patients with AF and in controls was investigated. Using the maximal standardised uptake value (SUV) of FDG-PET/CT, the authors observed a greater inflammatory activity of EAT in AF patients than in controls ($p < 0.001$).[271] Additionally, EAT SUV was significantly greater than peripheral subcutaneous adipose tissue (SAT) and visceral thoracic fat for patients with AF and controls, with EAT SUV being the only independent predictor of AF.[271] This lends credence to the postulation of a strong role of inflammatory component in epicardial fat-mediated pathogenesis of atrial arrhythmia, more so as FDG-PET/CT is reflective of macrophage burden.

Noteworthy, epicardial fat, as an archetypical visceral adipose tissue, is characterised by an intense metabolic activity and functions as an endocrine organ, serving as a source of several cytokines and proinflammatory mediators. Accordingly, Mazurek et al[272], in an earlier publication, demonstrated the presence of tumour necrosis factor-alpha (TNF-α), interleukin (IL)-1β and IL-6, and monocyte-chemotactic protein (MCP)-1, respectively, in the secretome of EAT from the patients with CAD. Direct release of MCP-1 attests to the

contribution of cellular infiltrates to the inflammatory phenotype of EAT. MCP-1 is a member of the C-C (gamma, γ) chemokine family of chemoattractants and it induces migration and recruitment of monocytes, the precursors of macrophages, in response to inflammation.[273, 274] In addition to producing pro-inflammatory cytokines, EAT also secretes anti-inflammatory markers including adiponectin.[275] In AF and obesity, it is plausible to speculate that the natural balance between pro- and anti-inflammatory cytokines of EAT becomes distorted and tilts in favour of a pro-inflammatory state. Indeed, reduced secretion of gelsolin (an anti-inflammatory adipokine) was has been reported in EAT of patients who went on to develop post-op AF after CABG.[276] In addition to its anti-inflammatory role, gelsolin is a negative regulator of the L-type Ca^{2+} channel, such that its deficiency has been associated with increased propensity for AF in a mouse model.[276]

Further, there is evidence to suggest involvement of EAT in activation of inflammatory cells. In patients with acute coronary syndrome, greater expressions of NOD-like receptor protein 3 (NLRP3), caspase-1 and pro-IL-1β, thereby indicating induction of NLRP3 inflammasome pathway.[277] Whether activation of this pathway is involved in induction of inflammation during EAT-mediated atrial remodelling remains to be elucidated. The lipotoxic nature of the resulting interstitial milieu has huge implications for induction of adipose tissue inflammation and a resultant higher propensity for the creation of AF substrate.

1.3.7.4 Atrial Myocardial Fibrosis and Epicardial Fat

The data on involvement of atrial fibrosis in EAT-induced structural remodelling are very limiting. Using an *ex vivo* organo-culture of rat atria with the secretome of EAT and

subcutaneous fat, Venteclef et al[278] demonstrated marked induction of global and interstitial fibrosis in rat atria pre-treated with the secretome of EAT compared to controls but found no such observation in SAT-treated atria. This indicates that EAT is also capable of inducing fibrosis and a resultant structural remodelling of the atrial myocardium.

The TGF-β pathway is considered the most important profibrotic pathway, insofar as it can occur downstream or independently of the renin-angiotensin-aldosterone system (RAAS) activation.[125] TGF-β superfamily of proteins represents a distinct clad of proteins that act via receptors with serine/threonine kinase properties to elicit a range of functional changes, including growth and differentiation. A subfamily of these containing the TGF-βs (TGF-β1, 2 &3) and the activins are particularly involved in fibrosis.[278] Interestingly, observation of abundant secretion of activin-A was made in the secretome of EAT in an *ex vivo* model, which was not the case for subcutaneous fat.[278] In the same study, supplementation of the organo-culture medium with recombinant activin-A reproduced the pro-fibrotic effects of EAT, which was blunted when the atrial tissues where pre-treated with activin-A-neutralising antibody.[278] This demonstrated that activin-A may mediate the pro-fibrotic actions of EAT on the atrial myocardium. Additionally, the same authors found significant expressions of matrix metalloproteinases (MMPs 1, 2, 3, 8, 9, and 13) in EAT as compared to peripheral adipose tissue.[278] Importantly, these molecules play key regulatory roles on extracellular matrix (ECM) remodelling, including matrix turnover, chamber dilatation, and basement membrane components. Increased activity of MMPs is tightly linked with heightened collagen fibres deposition, reactivity of fibroblast, and fibroblast-myofibroblast transition.[121]

1.3.7.5 Fatty Infiltration into the Left Atrium

Fat cell infiltration is a newly described histological substrate shown to be involved in formation of atrial substrate. In a seminal paper, Mahajan et al[182] provided an experimental demonstration of abundant deposition of fat in the epicardium and intra-atrial fat cell infiltration in an ovine sheep model of obesity on histology. Fatty infiltration was more significantly seen in the obese sheep compared to the age-matched controls and was shown to be more profound in the posterior LA and less in LA appendage, correlating with regions of reduced endocardial voltage.[182]

Clinical investigations of the phenomenon have been hampered by the limitation in available imaging modalities. Nonetheless, with the evolution of the Dark-blood DIR-prepared Fat-Water-separated sequence MRI imaging method, Tereshchenko et al[279] provided preclinical evidence for fatty infiltration of the atrial septum in the PRIMERI study. By evaluating 90 patients with structural heart disease, the authors found that infiltrated intra-atrial fat area was a significant and an independent predictor of a 10-year risk of AF risk based on the ARIC AF risk score (p=0.037).[279] Surprisingly, neither BMI nor total EAT area significantly predicted higher AF, thus, underscoring intra-myocardial fat in the left atrium as an evolving risk factor of pre-clinical AF.[279]

Rather than just being a mere histological observation or imaging assessment, infiltrated epicardial fat may underlie the formation of AF substrate. EAT adipocytes secrete a myriad of cytokines with the ability to alter the functional and structural properties of the atrial myocytes. Fatty infiltrates may exaggerate the paracrine or juxtacrine effects of EAT secretome atrial myocardium, allowing for a more direct modulating effect on the cardiac myocytes.[120] Furthermore, fatty infiltration can also allow for closer and somewhat direct

cellular crosstalk between fat cells and intramyocardial fibroblasts. Consistent with this, Venteclef et al[278], in a paper on atrial organo-culture, made an important observation of marked myocardial fibrosis in regions with EAT infiltration, with visible fibrotic fibres seen the interface between adipose and myocardial tissues. This was further confirmed by observations of association between AF and fibrosis of subepicardial fatty infiltrates in human and sheep models.[280]

Infiltrating epicardial fat may represent a novel and unique structural remodelling of the atria, which as noted in prior section plays an important role in the formation of permissive substrate for AF. It is well understood that fat acts as insulating tissue to the body incapable of conducting heat or electrical impulse.[281] Drawing from this premise, the presence of fat within the myocardial tissue can constitute local conduction blocks thereby leading to disorganization of the conduction waveforms and discontinuity in impulse conducting pathways/routes, behaving just like fibrotic fibres.[282] Intriguingly, Murthy et al[283] observed a significant and independent correlation between infiltrating fat and P-wave fragmentation in patients with paroxysmal AF and those at risk of AF. Abnormal P-wave indices are known to predict occurrence of paroxysmal AF. For example, abnormal P-wave morphology was independently associated with increased risk of non-sudden cardiac death and AF development.[284, 285]

1.3.7.6 Autonomic Tone Dysfunction and EAT expansion

Catecholamine excess and neurohumoral cascade activation are amongst the mechanisms purported to be responsible for the pathogenesis of AF.[64, 166, 286] These mechanisms are tightly linked to a dysfunctionality of baseline autonomic tone. Interestingly, epicardial fat is very

rich in ganglionated plexi, and thus has triggered an important postulate that it may negatively modulate the autonomic ganglia thereby enhancing a dysregulation of the autonomic tone which in turn may precipitate the episodes of AF.[287] Consistent with this, Muhib et al[288] reported some novel and intriguing findings from a hospital-based cohort with idiopathic hypertrophic cardiomyopathy and AF, showing a significant association between lower time-domain measures of heart rate variability (HRV) and the increase in EAT area. Additionally, these HRV measures also significantly decreased in patients with prevalent AF compared to AF-free controls, a relation that persisted after correcting for confounders.[289] In another study, both heart rate turbulence (HRT) and HRV parameters were investigated in two populations of EAT thickness.[290] In the same study, significant correlations between depression of these measures of cardiac autonomic functions and higher thickness of epicardial fat were noted.[290]

HRV and HRT are important and reliable 24-Holter ECG indices that indicate heart autonomic balance.[289, 291] HRV is an indirect measure of autonomic functions and reflects its influence on the sinoatrial node, whereas HRT is an indicator of baroreceptor sensitivity and is dampened in patients with reduced baroreceptor-cardiac reflex activity.[289, 292] Both HRV and HRT have been shown to predict AF and post-operative AF.[293-295] It may be possible that the secretome of EAT impact autonomic neurones that make up the GP richly embedded in the fat depot. In fact, a new report has implicated cardiac fat pads in abnormal autonomic neural remodelling.[296] Using canine model of AF, the authors demonstrated differential expression levels of long non-coding RNA (lncRNA) molecules involved in regulation of neural development, migration, neurodegenerative disorders, between patients with AF and SR controls.[296] They further showed that these lncRNAs could actually produce pro-arrhythmic effects, with genetic ablation of TCONS_00032546 shortening the atrial effective

refractory period thereby increasing AF vulnerability, whereas silencing of TCONS_00026102 prolonging ERP and prevented AF.[296] Thus, it may offer a potential mechanism to explain the increased propensity for AF due to epicardial fat expandability.

1.3.7.7 Electrical Remodelling with EAT Expansion

1.3.7.7.1 AF Triggers

Events originating as ectopic or focal discharges (involving EADs and DADs) and re-entrant circuits are thought to serve as initiating mechanisms for AF. Interestingly, Lin et al[297] showed in an *in vitro* model of LA myocytes an increased DAD amplitude after co-incubation with rabbit EAT adipocytes. In the same study, EAT significantly increased the incidence of triggered beats induced by isoproterenol, highlighting the importance of focal ectopy in EAT-induced arrhythmia.[297]

As noted earlier, early afterdepolarizations (EADs) are caused by mechanisms that prolong action potential duration (APD), including inhibition or loss of the ultra-rapid I_{Kur} and uncontrolled activation of $I_{Na\text{-}Late}$, which allow time for $I_{Ca\text{-}L}$ to recover from inactivation.[298] Indeed, co-incubation of LA cardiomyocytes with EAT adipocytes for few hours is shown to increase $I_{Na\text{-}Late,}$ with a concomitant increment in $I_{Ca\text{-}L}$, and 90% APD. Adipocytokines from EAT have also been shown to decrease I_{Kur} after 18 hours of incubation of myocytes with EAT secretome.[298]

1.3.7.7.2 AF Electrical Substrates

Mechanisms causing shortening of the effective refractory period (ERP) and conduction slowing form important substrates for AF. Consistently, a potential role of EAT in promoting

conduction disturbance of the atria has been investigated by several groups. In a study of 337 patients, atrial electromechanical interval was significantly increased in individuals with expanded EAT, and in after multivariable adjustment, EAT was found as an independent predictor of atrial electromechanical delay.[299] This denotes the involvement of reduction of voltage and lengthening of total activation time as potential mechanisms. This was recently corroborated by the work of Mahajan et al[300], which demonstrated pronounced voltage reduction and conduction slowing in regions adjacent to epicardial fat depots in obese patients. In the same study, left atrial EAT volume was found to demonstrate the best correlations conduction velocity, performing even much better than BMI ($r^2 = 0.31$ for LA EAT vs. $r^2 = 0.22$ for BMI).[300]

1.3.7.7.3 Mechanisms Maintaining AF

While re-entry seems to be the currently favoured mechanism of AF, several clinical mapping correlates have been utilised and include high dominant frequency (DF) and complex fractionated atrial electrogram (CFAE) sites.[156, 157, 160, 301] CFAEs are recognised as regions characterised by wave breaks and fusion which are associated with slow conduction and pivot activation.[164, 175] This repetitive and dyssynchronous nature of wave propagations of CFAE regions make them an important substrate for the perpetuation of AF. DF, on the other hand, are described as AF drivers with very high activation rate and that are central to a focal-firing rotor or local re-entry circuit.[160, 175] It is interesting that high DF sites have been shown to correspond to sites with larger EAT volume in both paroxysmal AF and persistent AF patients.[302] But data from the same study showed no relation between EAT and CFAE.[302] However, in another study, EAT volume and CFAE sites were independently associated with

AF, and significantly correlated with each other.[303] We have also demonstrated that EAT demonstrate correlation a better correlation with LA electrogram fractionation than BMI (r^2 = 0.55 for LA EAT vs. r^2 = 0.36 for BMI).[300]

1.3.7.8 Stroke and EAT Expansion

Stroke and thromboembolic events are a major complication in AF, and are associated with poor outcomes, increased need for thromboprophylaxis and excess deaths in AF patients.[21] The risk of stroke in AF is inhomogeneous and is conferred by comorbidities including ageing, coronary artery disease (CAD), hypertension (HTN), congestive heart failure and prior stroke which tend to act in additive fashion. Whether adiposity may increase the risk of stroke in AF is still under-investigated. In a prospective cohort of 190 patients, EAT thickness significantly predicted the incidence of cardiovascular events and EAT >6 mm was associated with significant reduction in CV event-free survival.[304] EAT was also shown to correlate with CHA_2DS_2-VASc score, though this was lost upon the addition of co-variates in the analysis.[304] Consistent with this, Akdag et al[305] found significantly increased EAT in nonvalvular AF patients at risk of stroke defined by a high CHA_2DS_2-VASc score. More recently, increased abundance of EAT volume was reported in AF patients who developed stroke and total EAT detected as an independent predictor of excess of stroke occurrence after AF diagnosis.[305]

Endothelial damage or dysfunction is an important determinant of a hypercoagulable state and is often present in AF. Molecules like soluble cell adhesion molecule 1 (sICAM-1) and von Willebrand factor (vWF) are important markers of endothelial injury and are both elevated in AF, predicting future development of stroke.[306] In a recent study, Girerd et al[306],

investigated the association of global or local EAT with markers of endothelial dysfunction in 49 AF patients. They found significant correlation between posterior EAT, located between the mid LA and the oesophagus (LA-ESO) or thoracic aorta (LA-ThA), and sICAM-1 and vWF in both local LA and peripheral vasculature independent of BMI and LA volume.[306] Whether this data provides a mechanistic insight into the potential role of EAT in thrombogenesis and risk assessment of patients for stroke in AF requires further studies.

1.4 Sudden Cardiac Death – A Background

Sudden cardiac death is a major public health issue, contributing to a significant proportion of cardiovascular deaths worldwide. SCD is best described by the rule of 50: 1, it contributes to over 50% of CV deaths; 2, in more than 50% of patients, SCD is usually the first presentation of a cardiac event; and 3, it leads to approximately 50% of years of potential life lost to heart disease as it occurs mostly during the most productive years of the victims.[14, 307-309]

The understanding of SCD is recently been appreciated despite a long history of the disease. In fact, the sudden nature of SCD was known and reported well before the time of modern medicine and molecular and genetic advancements. SCD has been described since the days of Hippocrates; in the Aphorism II, 41, around 400 BC, Hippocrates noted that individuals with syncope were more likely to die suddenly than those without. It would later be described by Da Vinci in the 1490s as occurring in victims of "shrunken and withered" coronary artery, and by Lancisi, in 1706 under the request of Pope Clement XI, as an epidemiological undertaking on sudden death, where he clearly linked SCD to cardiac hypertrophy and valvular diseases.[310]

1.4.1 Definition of SCD

The definition of SCD is best conceptualized by understanding "cardiac arrest", defined as: the cessation of mechanical function or activity of the heart, evidenced by termination of both cardiopulmonary and systemic circulations. Cardiac arrest could be of cardiac origin or non-cardiac origin, described in **Table 1**. Thus, SCD is temporally defined as a natural death from "sudden" cardiac arrest (SCA) in patients without known cardiac abnormality, occurring within an hour of onset of symptom (witnessed) or 24 hours in an unwitnessed case.[309, 311] Importantly, this takes into account three key elements of SCD: natural, rapid, and unexpected nature of the death.

Despite this definition, conflicting views surrounding SCD still exist among investigators, especially regarding the "1-hour" component. These differences can be settled by considering SCD in four perspectives: 1, its prodromes; 2, its onset; 3, cardiac arrest; and 4, biologic death.[309, 310, 312] The *prodromes* are a body of cardiovascular signs and symptoms, including chest pain, palpitations, dyspnea and fatigability, preceding and predictive of imminent cardiac event, but not specifically SCA. Because of huge individual variability, some victims do not experience *prodromes* at all. To be characterized as prodromal of SCD, their *onset* must occur suddenly and must precede the *onset* of *cardiac arrest* – within the 1-hour *onset* of terminal event that precipitate *cardiac arrest*. The *biological death* component refers to the consequence of a *cardiac arrest*, and it denotes the presence of irreversible damage caused by pathophysiological process that will ultimately lead to death. It is often where controversies arise as it is included in the 1-hour definition because, with prompt intervention and life support, death can be prolonged for few more hours to days despite there been an irreversible damage.[310, 312]

1.4.2 Public Health Burden of SCD

SCD is associated with a significant cost to the healthcare systems, communities and/or individuals alike. Between 2003 and 2012, Damluji et al[313] showed a linear increase in hospitalizations and hospitalization-associated costs accruing to SCA, mostly due to automatic implantable cardioverter defibrillators (ICDs) use, hypothermia and oxygenation therapy (odds ratio 1.3 to 2.4). On a personal level, premature death from SCD accounts for 50% of years of potential life lost (approximately 2 million years in men and 1.3 million years in women) from cardiac disease.[14, 308, 309, 314]

Accurate estimation of the public health burden of SCD remains a significant challenge in cardiovascular epidemiology. By extrapolating from the Seattle Emergency Service system, Cobb et al[315] estimated an annual incidence of 184,000 of treated out-of-hospital cardiac arrest (OSCA) events per year in the United States from 1979 through 2000. Low estimates like this are usually when coronary artery disease is the primary aetiology of SCD. Interestingly, however, up to 460,000 events per year are reported when all factors contributing to SCD are taking into account.[14, 309] The latest statistical textbook of the American Heart Association (2019) puts the annual rates of SCD at 366,494, representing more than 50% of all recorded deaths due to heart disease in the USA.[14] Further, a recent prospective multicentre study demonstrated incidence of 84 OSCA cases per 100,000 in 27 European countries, which, at a survival rate of 10%, equates to 131,544 SCD events per year for the 174-million population.[316] The true SCD would even be higher when in-hospital SCA (ISCA), currently at 55.5% of 348 368 patients managed in teaching hospitals and 58.8% of among 376 035 managed in nonteaching hospitals in the USA alone, is taken into account.[14]

1.4.3 Mechanism of SCD

The general understanding is that the development of SCD requires two important components: 1, an established substrate (substrate-based cause); and 2, an arrhythmic trigger. The substrate-based causations are conditions that predispose to SCD; they consist in pathological myocardial, vascular, or molecular adaptations that culminate in arrhythmic expressions.[317] A majority of these substrates are created in CAD settings; examples include the plaque transition, acute coronary syndromes, and ischaemia modules in patients.[317, 318] It is crucial to note that, in non-ischaemic conditions, the substrates are starkly different. Mechanisms mediating the clinical expression of predisposing SCD substrates constitute the triggers; these are identified as pro-arrhythmic cascade events discussed below.[318-322]

1.4.3.1 Lethal Ventricular Tachyarrhythmias

The initial rhythm leading to sudden cardiac arrest and death typically starts as a ventricular tachyarrhythmic event, such as ventricular fibrillation (VF) and pulseless or sustained ventricular tachycardia (pVT). The incidence of tachyarrhythmic mechanism is documented in up to 80% of SCD cases.[309] For example, data from the Resuscitation Outcomes Consortium investigators demonstrated rates of 38%, 60% and 79% of VF or pulseless pVT as witnessed by emergency-medical service personnel, a bystander and when a bystander applied an automated external defibrillator, respectively.[323] The true rates might even be higher given the deterioration and progression to non-shockable events.

The mechanism of pVT or VF bears semblance with atrial tachyarrhythmias, such as AF. In fact, SCD is associated with AF, and in a recent meta-analysis of 8401 AF patients

and 67,608 controls in sinus rhythm, 2.22-fold increased risk of SCD was seen in AF patients compared to SR even persisting after multivariable adjustment.[324] Like in AF, ventricular tachyarrhythmias require electrical trigger events acting on vulnerable ventricular substrates. Importantly, induction of fibrotic scarring, inflammation, abnormal connexin proteins, abnormal Ca^{2+}-handling and membrane currents, and molecular and cellular maladaptations have all been implicated in substrate the formation of re-entrant tachyarrhythmias.[322, 325-329]

1.4.3.2 Non-tachyarrhythmic Mechanisms

After the initial arrhythmic events during SCA, it is known that pVT/VF degenerates into non-tachyarrhythmic mechanisms, including asystole and pulseless electrical activity (PEA). PEAs are higher in younger patients and in IHCA victims.[320] Additionally, asystole and PEA are more prevalent in non-ischaemic SCD cases than in ischaemic cases.[330] There is also evidence that bradyarrhythmia can lead to SCD, especially severe episodes capable of causing loss of circulation (both cerebral and gross organ perfusions). Noteworthy, there is bidirectionality in deterioration of arrhythmic mechanisms, in that, just as tachyarrhythmias can degenerate into non-tachyarrhythmic events, these non-shockable arrhythmic can spontaneously change into shockable VF or pVT.[310, 312]

1.4.4 High-risk vs Low-risk Risk Profiling – Scaling the Bottleneck in SCD Estimation

Reduction of SCD burden is wholly dependent on the accurate identification of at-risk populations, but this is currently plagued by the available risk stratification tools. These tools adopt risk profiling of populations that are based on coronary heart disease, ischaemic heart

disease, congestive heart failure, inherited channelopathies, and cardiomyopathies, which traditionally have high incidence rates of SCD.[312] Despite recent evidence purporting to the decline in prevalence of these *high-risk* cohorts and associated deaths, the occurrence of SCD has not demonstrated consequent decreasing trend, see **Figure 4**.[14, 309, 312] Moreover, data has shown the general absolute events that occur per year are far greater than accounted for by the traditional cardiac risk factors and a history of heart disease, see **Figure 2**.

Further, clinical risk profiling for implantable cardioverter defibrillators (ICD) prophylaxis using ejection fraction (EF) less than 30% has not yielded promising results. For example, retrospective assessment of LVEF by Stecker et al[331] in victims of SCD demonstrated severe LV dysfunction (LVEF ≤35%) in only 30% patients, indicating that up to 70% of the victims would not have qualified to ICD prophylaxis. Thus, investigation of new risk markers for SCD in the general *low-risk* populations is warranted now more than ever.

1.4.5 Contagion of SCD in Excess Adiposity

A growing postulate posits that the increasing burden of SCD may be driven by the rise in novel cardiovascular risk factors, such as obesity, which is indeed a global epidemic, see **Figure 4**. And as earlier noted, the risk of *high-risk* SCD features are greatly increased in obese individuals compared to normal weight counterparts.[4, 7, 332-336] Recent findings from community-based population studies have reported independent association between SCD and obesity.[337, 338] More intriguingly, obesity-mediated cardiomyopathy has even been implicated as the most common non-ischaemic cause of SCD, suggesting involvement of adiposity in SCD development.[339]

1.5 Stable Obesity or Weight Fluctuation – which is worse?

Data showing the perils of increasing adiposity during weight gain, such as expansion of EAT, is just beginning to gain traction. The risks of arrhythmogenic conditions like AF[261] and SCD[337] are increased in obese patients compared to normal weight subjects. This may be explained in part by the fact that obesity also promotes traditional clinical correlates, such as metabolic syndrome, sleep apnoea, heart failure, acute coronary syndromes, and valvular heart disease.[5, 7, 335] However, the rising burdens of cardiac arrhythmias are more than can be explained by the mere presence of these comorbid states alone. With the induction of low-grade systemic pro-inflammatory state, renin-angiotensin-aldosterone system activation and pro-fibrotic signalling in the obese, it is very likely that obesity directly impacts cardiac remodelling and arrhythmic substrates.[340] Clinical associations are reported with markers of atrial dysfunction[260], atrial enlargement[260], chronic inflammation[340], and neurohumoral dysfunction, all of which are strongly linked to atrial fibrillation (AF).Yet still, there is a lot we do not know about the excess fat/AF risk relation.

In preclinical models, several defining links have been uncovered for the pro-arrhythmogenic role of obesity. These include: increased fibrosis and gross endocardial remodelling found in a chronic ovine model[182]; marked expression of pro-fibrotic cytokine after induction of short-term obesity and overweight in sheep[181]; reduction of Cx40 protein and P-wave duration leading to sustained atrial arrhythmia in rats following high-fat feeding (8 weeks)[341]; abbreviation of PV refractoriness with subsequent increased vulnerability to AF in porcine atria after 18-week high-fat diet[342].

Interestingly, these data show that therapeutically targeting adiposity could mitigate arrhythmogenic substrate and help improve AF management. Indeed, this has been tested in several clinical trials. For example, Abed et al[334] showed that weight reduction with intensive cardiometabolic risk factor management reduces symptomatic burden and severity of AF and produces additional cardiac remodelling. Nevertheless, there are important questions surrounding the long-term sustainability of weight loss programs. Noteworthy, weight loss is associated with several compensatory mechanisms, including reduced resting energy expenditure[343], increased appetite, and both short- and long-term changes in appetite regulators (e.g., leptin, ghrelin, and cholecystokinin)[344], that ultimately lead to weight relapse in patients. Pathak et al[345] prospectively tested this concept in patients who underwent catheter ablation for AF in the LEGACY (*L*ong-term *A*ffect of *G*oal-directed weight management of *A*trial fibrillation: a 5-*Y*ear follow-up study) trial. The authors demonstrated that long-term weight loss significantly reduces the burden of AF and echocardiographic left atrial substrates. However, they found that the beneficial effects of weight loss were counteracted by >5% weight fluctuation (weight loss/gain cycles) in patients.[345] More importantly, the effect of weight fluctuation was shown to be independent of baseline BMI, with over 2-fold greater risk AF recurrence predicted by >5% weight fluctuation (HR: 2.06, p=0.02).[345] Thus, this data highlights the potential pro-arrhythmic consequences of fluctuating weight. The question remains whether the substrate for AF in stable obesity differs from weight fluctuation. If so, it is not known which is worse as knowledge of this will drastically improve treatment options available for patients struggling with weight management.

1.6 TABLE

Table 1. Definition of Cardiac Arrest in Context

Terms	Description	Reversibility
Sudden cardiac death	Sudden, irreversible cessation of all biological, biochemical and biomechanical functions as a consequence of cardiac arrest	Not reversible
Cardiac arrest	Cessation of cardiac mechanical function that may lead to death in the absence of reversal by a prompt intervention. May be: - Medical (e.g., cardiac, anaphylaxis, asthma or DI bleed) - Traumatic - Drug overdose - Drowning	Rarely reverse spontaneously. Reversibility determined by: - Mechanism of arrest - Clinical setting, and - Prompt return of circulation
Cardiovascular collapse	Characterised by sudden loss of effective blood flow because of cardiac and/or peripheral vascular factors	May reverse spontaneously: - Neurocardiogenic syncope - Vasovagal syncope or May require interventions: - Cardiac arrest

1.7 FIGURE LEGENDS

Figure 1. Mechanism of Excitation-contraction Coupling of Cardiomyocytes

Figure 2. Mechanisms of Focal Ectopic Activities

(**a.**) Normal action potential phases with a resting potential around -80 mV, Na^+-induced upstroke, plateau phase mediated by Ca^{2+} entry through both LTCC and RyR2, early repolarisation and late repolarisations. (**b.**) Formation of early afterdepolarisations occurring during the plateau phase of AP, and sustained ectopics. (**c.**) Formation of DADs during late phase (4) of repolarisation. Insufficient diastolic Ca^{2+} leak may cause membrane oscillation but not enough to cause AP; but when this is strong enough, full AP may be generated leading to DAD and ectopics when sustained.

Figure 3. Mechanisms of Re-entry

Figure 4. The Incidence of SCD and Absolute Annual Events in Population Subsets

With increasing incidence, based on subgroup profiling, a decrease in proportion of the total sudden death burden is seen. This effect relates to the population impact of known evidence-based outcomes of various prevention therapies, and it highlights the challenge of the low-risk, high-numbers subsets. (Modified from Myerburg RJ, Junttila MJ. Sudden cardiac death caused by coronary heart disease.)

Figure 1. Mechanism of Excitation-contraction Coupling of Cardiomyocytes

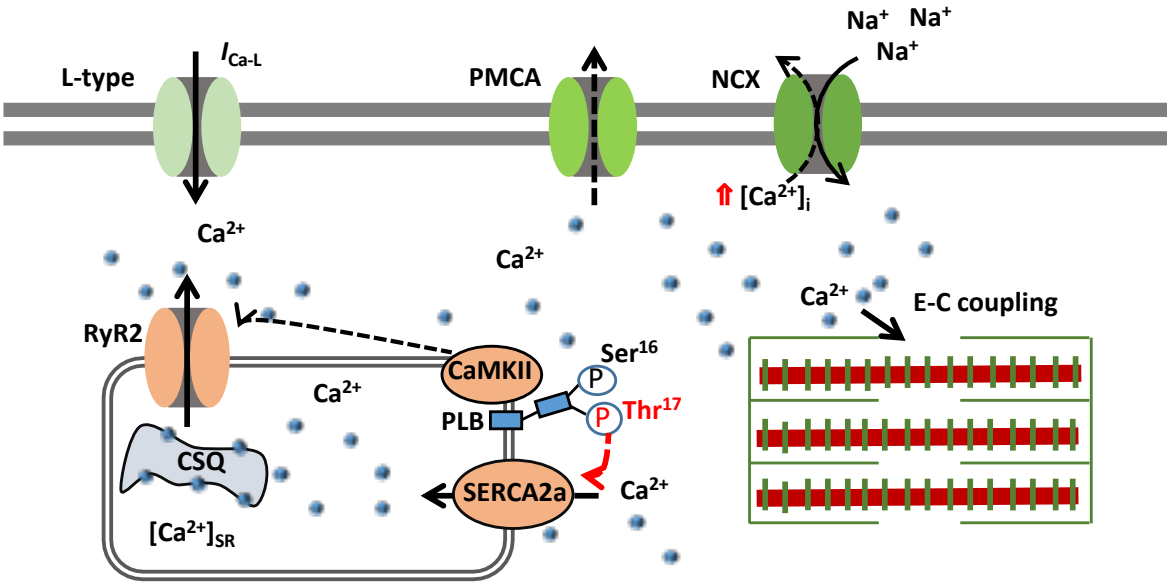


Figure 2. Mechanisms of Focal Ectopic Activities

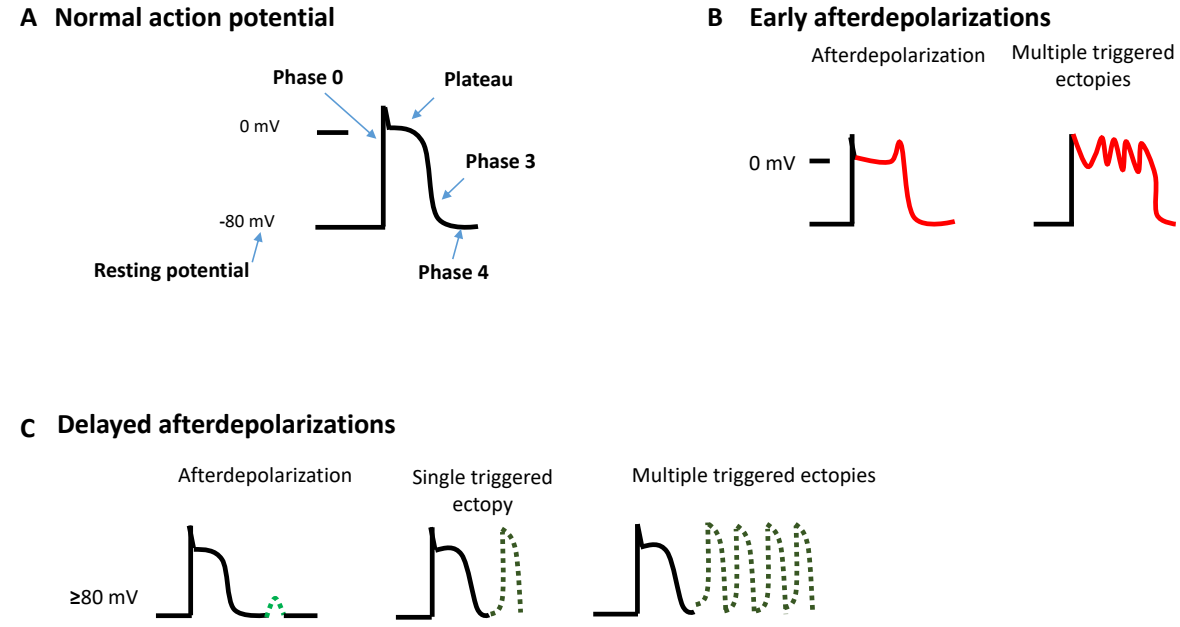


Figure 3. Mechanisms of Re-entry

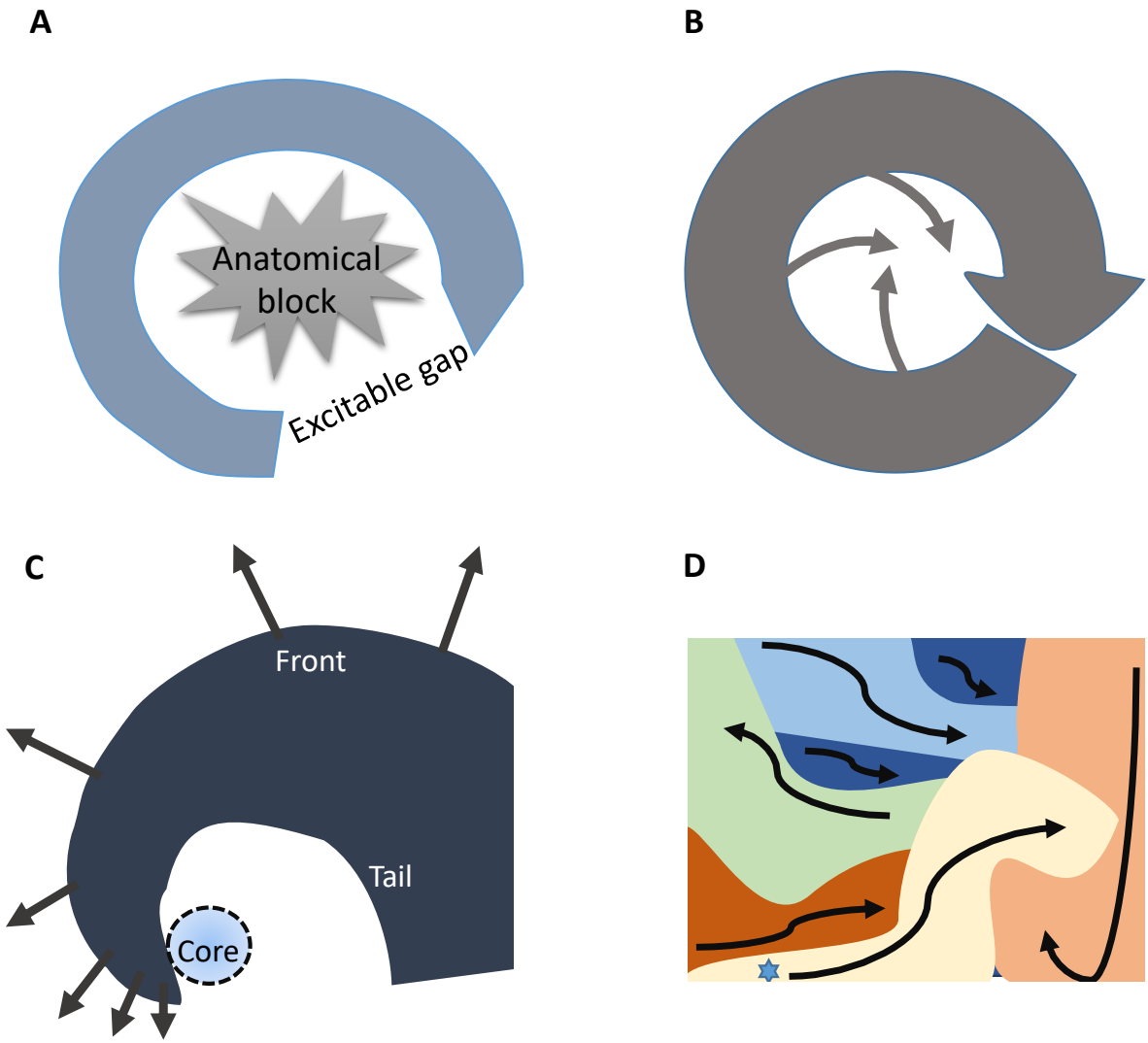
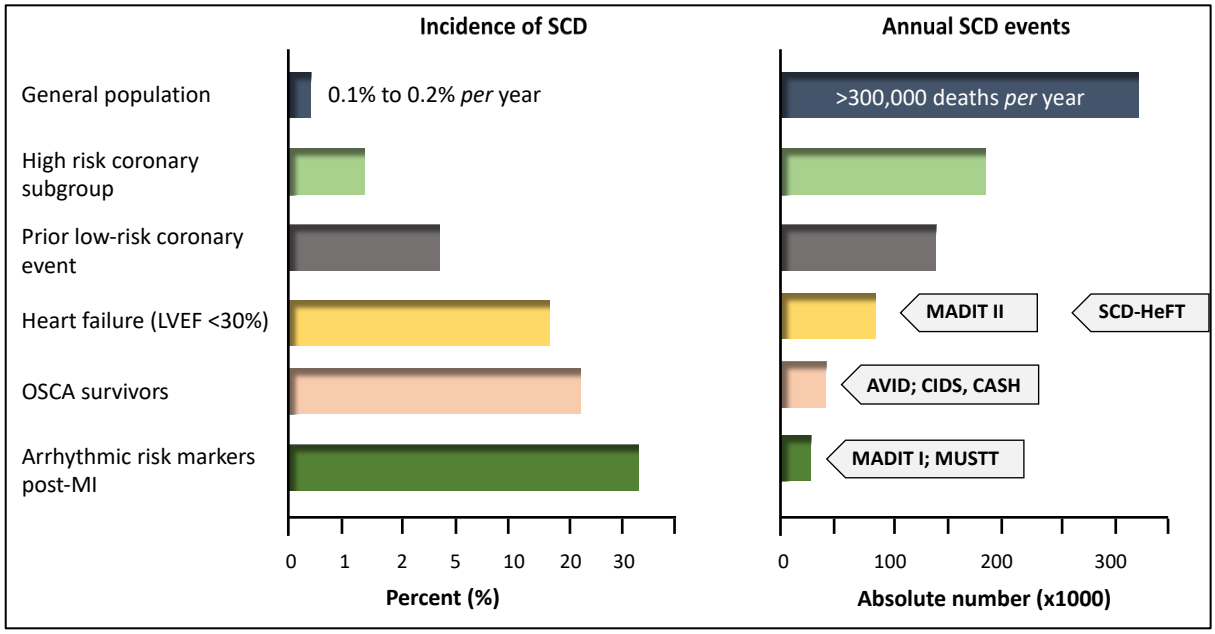


Figure 4. The Incidence of SCD and Absolute Annual Events in Population Subsets



2. Chapter Two

Galectin-3 as a Predictor of Atrial Fibrillation – A Meta-analysis

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2.1 INTRODUCTION

Evidence from a body of clinical and experimental studies have established fibrosis as the hallmark of structural remodelling that forms the substrate for atrial fibrillation.[125] Fibrosis is associated with greater risk of cardiac arrhythmias and shown to predict poorer prognosis post-catheter ablation. Despite this, identifying modifiable risk correlates of fibrotic remodelling that would help detect patients at risk of future AF remains a challenge.

More recently, studies have suggested that galectin-3 (Gal-3) may be important for risk stratification and prognostication of AF.[346, 347] Gal-3, a β-galactoside-binding lectin, is shown to be involved in important regulatory functions, such as cell adhesion, inflammation, and fibrosis.[348] Consequently, Gal-3 is reported to have important prognostic value in traditional risk factors for AF. Indeed, Gal-3 is associated with fibrotic remodelling in heart failure[349], and high serum Gal-3 is correlated with increased risk of incident of heart failure[348, 350] and mortality in several epidemiological studies[348]. It is notable that diminished atrial electrical and fibrotic remodelling were reported following therapeutic targeting of cardiac Gal-3 in an experimental model.[351] In the same study, Gal-3 inhibition was associated with increased AF termination and reduced AF burden, indicating that Gal-3 may be a druggable upstream target for the prevention of AF.[351] Nevertheless, the nature and strength of the relation of this fibroinflammatory biomarker with AF prevalence and incidence have not been properly defined. Moreover, it not well described whether Gal-3 could influence the prognosis of catheter ablation.

In the present study, we hypothesised that plasma Gal-3 would be increased in patients with AF and that it be a predictor of recurrent arrhythmias after catheter ablation for AF. Thus, we aimed to: (1) Investigate the relation between Gal-3 and the presence of AF;

(2) Evaluate its association with the risk of incident AF in prospective or retrospective cohort studies; and (3) Characterise association between baseline or pre-ablation Gal-3 levels and the recurrence of AF after catheter ablation.

2.2 METHODS

2.2.1 Search Strategy

This meta-analysis is being registered on **PROSPERO (ID: 129278)** and conducted according to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) statement. References were identified through online database searches done on PUBMED, EMBASE, Ovid MEDLINE and the Core Collection of Web of Science. Searches were conducted from inception of each database through 16 March 2019; the following keywords were used: (Galectin-3 OR Gal-3) AND (Atrial Fibrillation OR AF). The retrieved papers were exported to and sorted by EndNote X9.1 software.

2.2.2 Inclusion and Exclusion Criteria

Review authors Thomas A. Agbaedeng and Mehrdad Emami carried out the screening of references for eligibility and inclusion, with any discrepancy resolved by consensus. Papers were retrieved based upon the titles followed by the scrutiny of their abstracts and full-texts to ensure nothing was missed. Papers were first excluded based on the following criteria: (1) non-English publications; (2) Whether they were conference reports and abstracts that were not yet published; (3) editorials and letters to the editor; (4) case reports and case series; and (5) duplicate publications. The reference lists of review articles were searched for relevant

original papers and excluded thereafter. In the next stage, the full-texts of the references were properly perused, with non-relevant studies excluded thereafter. Finally, we included studies if they reported on: (1) Serum Gal-3 levels in patients with and/or without prevalent or incident AF; (2) Odds ratio (OR), relative risk (RR), or hazard ratio (HR) of association of Gal-3 with AF; (3) AF prevalence or incidence in different quartiles of Gal-3; (4) Serum Gal-3 levels in patients with or without recurrence following catheter ablation of AF; and (5) OR, RR or HR of association between Gal-3 with post-ablation AF.

2.2.3 Study Selection and Data Extraction

The study selection and data extraction were done by review authors using an a priori determined set of guidelines. The following outcomes and data were collected: (1) Study authors; (2) Publication year; (3) Country of publication; (4) Study design; (5) Mean age of participants; (6) Participants; (7) Study endpoints; (8) Follow-up duration; (9) AF incidence, prevalence, recurrence after catheter ablation; (10) Serum Gal-3 level; and (11) Risk estimates (OR/RR/HR).

2.2.4 Risk of Bias and Quality Assessment

The methodological qualities of the included studies were assessed on the bases of the study design using the Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomised studies in meta-analyses. The scales for case-control studies and cohort studies were used to assess the quality of case-control and cohort studies, respectively. The following perspectives were used to evaluate quality of cohort studies:

1. Selection of study groups,

2. comparability of these study groups, and
3. The ascertainment of the outcome of interest.

And the following for case-control studies:

1. The selection study groups,
2. The comparability of the participant groups, and
3. The ascertainment of the exposure of interest.

The judgement of the studies was done using a "Star System" and computed as quality scores ranging from 1 to 9. A quality score of 1 indicated an extremely poor methodological design and a score of 9 was indicative of a very good quality.

Randomised controlled trials were assessed for risk of bias (RoB) using the Cochrane Risk of Bias Assessment Tool for Randomised Studies of Intervention (the Cochrane Collaboration). We assessed the RoB in RCTs based on seven domains through which bias is likely to be introduced into these studies, namely:

1. Bias due to random sequence generation (*Selection Bias*)
2. Bias due to allocation concealment (*Selection Bias*)
3. Bias in blinding of participants and personnel (*Performance Bias*)
4. Bias in blinding of outcome assessment (*Detection Bias*)
5. Bias due to incomplete outcome reporting (*Attrition Bias*)
6. Bias due to selective reporting of outcomes (*Reporting Bias*)
7. Bias due to other sources (*Other Bias*)

2.2.5 Data Synthesis and Analysis

A random effects meta-analysis was conducted on the pooled results from the various citations using RevMan (The Cochrane Collaboration, Copenhagen). Two meta-analytic effects size types were used for the data analyses, namely: standardised mean difference (SMD) and risk ratios (RR). SMD typically measures the size of an outcome relative to the standard deviation (SD) of the outcome, thus, reflecting the real differences in the variability of the measured outcomes. RR's were pooled from studies that conducted multivariable analysis, and this was done to show independent association with AF as well as a prediction of AF. We used the most adjusted model, which corrected for the following clinical correlates: age, sex, heart failure, type 2 diabetes, hypertension, obstructive sleep apnoea, coronary artery disease, myocardial infarction, obesity, valvular heart disease, peripheral vascular disease, and stroke.

Serum galectin-3 was measured by enzyme-linked immunosorbent assay and pooled as mean plus/or minus standard deviation (mean±SD). The degree of heterogeneity of Gal-3 estimates across the studies was assessed by examination of forest plots, chi-squared (Chi^2) test and I-squared (I^2) statistic. The latter two provide numerical values for an assessment of heterogeneity, with a high Chi^2 relative to the degree freedom suggestive of variations in effect estimates and I^2 greater than 50% indicative of a considerable amount of heterogeneity ($p<0.1$ defined as the cut-off). Statistical significance was set at $p\leq0.05$.

2.3 RESULTS

2.3.1 Search Result and Synthesis of the Literature

The online database searches and supplementary searches conducted resulted in a total of 460 references. One hundred and twenty-three duplicate publications were removed and a further 320 references upon applying the exclusion criteria. Fifteen studies (13 observational and 2 randomised controlled trials [RCT]) met our inclusion criteria and were included in this review. A pictorial overview of the search strategy and selection methodology is shown as a flowchart in Figure 1.

2.3.2 Study Characteristics

A full description of the characteristics of the included studies are provided in **Tables 1 to 3**, including study designs, quality scores, demographics, methodology, study endpoints, follow-up, and participants.

The 15 included studies had a total of 13,736 participants (6,454 [47.0%] males and 7,282 [53.0%] females) from 9 countries (1 study from both Switzerland & Germany, 1 from Italy, 1 from Germany, 1 from China, 1 from France, 1 from the UK, 2 from Serbia, 3 from Turkey, and 3 from the USA). Of the 13 observational studies, 9 reported on pre-ablation (incident and prevalent) AF[346, 347, 352-358], 2 on post-ablation AF[359, 360], and 2 on both pre- and post-ablation AF[361, 362], respectively. There were 9 case-controls, 7 cohorts, and 1 prospective, community-based study.

In the RCTs, Wijk et al[363] reported on 2 trials (Trial of Intensified versus Standard Medical Therapy in Elderly Patients with Congestive Heart Failure [TIME-CHF] and Gruppo

Italiano per lo Studio della Sopravvivenza nell'Insufficienza Cardiaca [GISSI-HF]) and thus contributed to studies. Of the three RCTs, PROTECT study was a single-centre, prospective, randomized trial[364]; TIME-CHF was multicentre, multinational trial involving 15 centres in Switzerland and Germany[363]; and GISSI-HF were multicentre, randomized trial involving 357 centres in Italy[363]. The follow-up period ranged from 0.8 years in PROTECT study to nearly 4 years in GISSI-HF study (mean±SD: 2.0±1.7 years). In total, the RCT contributed 1,001 patients (82.9% males and 17.1% females) and average age of 72±8.5 years.

2.3.3 Risk of Bias and Study Quality

The methodological study quality was assessed using the NOS quality scale, running on a 1-9 scale. In the case-control studies evaluating AF presence and severity, the quality was judged to be average, ranging from 5 to 7, see **Table 1**. The two prospective cohorts that investigated AF incidence had high quality score (8 in both). For the 3 RCTs, RoB assessment yielded low to moderate risk of bias, see **Table 2 & Figure 2**. The quality of the studies on post-ablation AF was judged to be moderate (mean [±SD]: 5.5±1.3), with least being 4 and highest scoring 6, see **Table 3**.

2.3.4 META-ANALYSIS

2.3.4.1 Gal-3 and AF Presence

We identified seven case-control studies[352-355, 357, 358, 361] reporting on serum Gal-3 and the presence of AF with a total of 718 participants. Mean serum Gal-3 levels (±SD) was extracted from each study and pooled in a random-effects meta-analysis. There was a

significant difference between the levels of Gal-3 in patients with pre-existing AF and sinus rhythm (SR) controls, **Figure 3**. We found 0.58 ng/mL higher plasma Gal-3 in AF patients as compared to SR controls (SMD: 0.58, 95% CI: 0.38 to 0.78, p<0.0001). We could not further compare the association of Gal-3 with AF presence due to lack of adjusted risk estimation in the studies. The largest contribution to the overall estimate was by Gurses et al[353] at 20.4%, followed by Begg et al[355] (16.9%), Wu et al[362] (14.8%), equally by Sonmez et al[352] and Selcoki et al[354] (13.9%), and Pavlovic et al[357] and Kornej et al[361] coming bottom (10.2% and 9.9%).

2.3.4.2 Gal-3 and AF Incidence

We identified 2 references[363, 364] reporting on prospective, randomized trials compared the incidence of AF in a dichotomous Gal-3 population (high vs. low). One of the references reported two RCTs, namely TIME-CHF and GISSI-HF, so there were 3 trials altogether. We found significant association between high Gal-3 level and AF incidence, **Figure 4**. High plasma Gal-3 associated with 57% increased odds of AF compared to low Gal-3 level (OR: 1.57, 95% CI: 1.15 to 2.15, p=0.005). GISSI-HF trial explained almost half the pooled risk estimate, with PROTECT and TIME-CHF trials contributing a quarter each, **Figure 4**.

Next, wanted to explore the relationship between continuous increment in Gal-3 and AF incidence. We found 2 large prospective, cohort studies[347, 356] that included 11,742 patients (1,435 incident AF cases) and pooled effect size estimates from the most adjusted models. Covariates adjusted for included: age (years), sex (male, female), height (metres), weight ($kg.m^{-2}$), systolic and diastolic blood pressures (mm Hg), antihypertensive medication use (yes, no), diabetes mellitus (yes, no), smoking status (current, former, never), history of

myocardial infarction (yes, no), history of HF (yes, no), total cholesterol (mg/dL), eGFR (mL/min/1.73 m^2), rs4644 genotype (CC, AC, AA), ln NT-proBNP (ln pg/mL), ln CRP (ln mg/L), and ln TnT (ln ng/L).[347, 356] Gal-3 associated with increased incidence of AF, with 1-unit increment in Gal-3 associated with 26% increased hazards of incident AF ([OR: 1.26, 95% CI: 1.08 to 1.48, p=0.004]; **Figure 5**).

2.3.4.3 Gal-3 and AF Severity

Three studies[346, 353, 358] provided data on Gal-3 assessment in patients with paroxysmal and non-paroxysmal AF (persistent and permanent AF). A total of 314 patients contributed to this analysis. When they were pooled, we found a significantly higher level of Gal-3 in patients with non-paroxysmal form of AF as compared to paroxysmal AF, see **Figure 6**. NPAF was associated with 0.51 greater SMD of Gal-3 as compared to PAF (95% CI: 0.28 to 0.73, p<0.0001). More than half of this is explained effect estimate from Clementy et al[346] (58.9% weight).

2.3.4.4 Gal-3 and AF Recurrence

Four studies[359-362] were identified that investigated relation of Gal-3 with catheter ablation outcomes. In the pooled analysis, we found that patients with AF recurrence had greater baseline plasma Gal-3 levels as compared to those without recurrence. Patients with arrhythmia recurrence had 0.96 higher SMD Gal-3 ([95% CI: 0.09 to 1.83, p=0.03]; **Figure 7**). Interestingly, we pooled the adjusted risk estimates, we found non-significant association between 1-SD increase in Gal-3 and risk of recurrence. According to **Figure 8**, although

there were 22% adjusted odds of AF recurrence per 1-SD Gal-3, this did not achieve statistical significance (OR: 1.22, 95% CI: 0.86 to 1.72, p=0.26).

2.3.4.5 Heterogeneity and Sensitivity Analysis

We evaluated statistical heterogeneity in the studies using Chi^2 (degree of freedom) and I^2-statistic. The pooled analysis for Gal-3 and AF presence showed only mild inconsistency in effect size estimates (Chi^2: 9.18, df: 6, p=0.16; I^2: 35%), **Figure 3**. In NPAF vs. PAF analysis, there was no evidence statistical heterogeneity (Chi^2: 0.69, df: 2, p=0.71; I^2: 0%), **Figure 6**. Similarly, no evidence of heterogeneity was found in the analysis of AF incidence ([Chi^2: 0.12, df: 6, p=0.94; I^2: 0%]; [Chi^2: 0.27, df: 1, p=0.60; I^2: 0%]; **Figures 4 & 5**). However, we found moderate to substantial heterogeneity in the rest of AF recurrence comparisons, see **Figures 7 & 8**.

2.4 DISCUSSIONS

2.4.1 Major Findings

Gal-3 is implicated in cardiometabolic risk factors like obesity and heart failure. Its role in the pathogenesis of AF has been suggested but not fully described. In this meta-analysis, we explore the relationship between plasma Gal-3 and AF, demonstrating that:

1. Plasma Gal-3 is significantly increased in prevalent AF compared to having no pre-existing AF
2. High Gal-3 associates with 57% greater risk of incident AF

3. For every 1-SD increase in Gal-3, patients are at 26% elevated risk of developing AF, even after correcting for baseline covariates
4. Patients with non-paroxysmal AF significantly increased levels of Gal-3 compared to paroxysmal AF

2.4.2 Mechanisms Promoting AF

AF is a heterogenous condition with several factors identified to promote its development. These factors are further classified as mechanisms responsible for AF initiation and mechanisms perpetuating the arrhythmia once initiated. AF triggers were first identified by Haïssaguerre et al[119] as ectopic activities originating from the pulmonary veins. In this seminal investigation, localization of ectopic foci in the PVs, by multielectrode catheter mapping, was observed in 94% of patients with paroxysmal AF (PAF), which were successfully ablated with radio-frequency energy. The author further demonstrated high efficacy of therapeutic targeting of these foci, with 62% patients shown to be free of AF recurrence after 8(±6) months of follow-up post-ablation.[119] These findings have been replicated by multiple investigators[365-367], with up to 86.1% freedom from AF recurrence reported in follow-up studies[365]. Additionally, AF triggers have been identified in other sites, such as: posterior wall of the left atrium; the superior vena cava; inferior vena cava; crista terminalis; ligament of Marshall; coronary sinus ostium; and interatrial septum.[368, 369]

Localized re-entrant and multiple wavelets[11, 94, 120, 205] are hypothesised to drive AF maintenance. These have been mapped to structural and morphological changes[94], electrical alterations[151], re-entry involving functional[370, 371] and anatomic[166] lines of block in the atrial tissue. Interesting, fibrosis is understood as the histological hallmark of structural substrate

for initiation and perpetuation of AF.[125] Data from the DECAAF study indicated that left atrial fibrotic substrate by delayed-gadolinium enhancement MRI independently predicts AF recurrence following ablation.[372] Others have further confirmed this finding, including association of fibrosis with cardiac and all-cause mortality and sudden cardiac death.[373-375] Taken together, these data show that full understanding of the pathophysiology of AF would require investigations of multiple pathways, including the role of biomarkers in substrate formation.

2.4.3 Galectin-3 and AF

Recent findings have implicated galectin-3 in the clinical link between fibrosis and development of atrial substrate for AF. In two large prospective cohorts, 1-unit increment in circulating Gal-3 was associated with 2.29- and 1.19-fold increased hazards of incident AF.[347, 356] In the Framingham Offspring Cohort, it was shown that this association becomes nonsignificant after correcting for traditional risk factors for AF, thus highlighting role of other modifiable risk factors in this picture.[347] Despite this, when we pooled the two cohorts together in a meta-analysis, we found elevated adjusted risk of AF per unit increment in the biomarker. We also found higher rates of AF incidences in high Gal-3 populations compared to low Gal-3 and associated Gal-3 with greater persistence of AF. We believe this meta-analysis provides robust evidence for association between Gal-3 and AF. We employed multiple study types, including case-controls, prospective cohorts, and randomized trials to evaluate this association.

2.4.4 Galectin-3 and AF: Role of Increased Adiposity

The association of Gal-3 and AF may be attributed to cardiometabolic perturbations. Circulating levels of Gal-3 are higher in patients with obesity[376], abdominal adiposity[377], dyslipidaemia[377], and hypertension[377]. Similar findings are reported in pre-clinical models. In obese rats, treatment with Gal-3 inhibitor modified citrus pectin (100 $mg.kg^{-1}$ per day) ameliorated adipocyte differentiation, adipose tissue inflammation, pericellular collagen deposition, prompting the authors to conclude that Gal-3 could play an important role in metabolic alterations resulting from obesity.[378] Increased Gal-3 levels are also associated with adipocyte dysfunction, contributing to insulin resistance , and cardiac lipotoxicity in obesity.

Galectin-3 is a β-galactoside-binding lectin mainly secreted by macrophages and to a lesser extent by adipocytes and fibroblasts.[379] We speculate that, during obesity, Gal-3 may mediate epicardial fat dysfunction and downstream atrial structural and electrical remodelling. Consistently, Gal-3 levels are reported to associate with diastolic dysfunction the morbidly obese[376]; in obese male Wistar rats (induced by HFD), Gal-3 is associated with fibrosis and inflammation[378]. This is further mapped to abnormal leptin, an adipokine secreted by epicardial fat, signalling; Gal-3 reportedly promotes leptin-induced deposition of collagen I and oxidative stress.[379] The most compelling evidence comes from a seminal investigation Takemoto et al[351]. The authors observed increased expression of Gal-3 in cardiac microcirculation of persistent AF than paroxysmal AF.[351] LA Gal-3 level was found to be an independent predictor of AF recurrence after catheter ablation. The authors also report reduction of proliferation of atrial fibroblasts *in vitro*, and amelioration of atrial enlargement, hypertrophy, fibrosis, and dominant frequencies in sheep, following Gal-3

inhibition.[351] These translated to reduction vulnerability to spontaneous AF and AF burden in the sheep model[380], thus underscoring a role in of Gal-3 in local atrial remodelling.

2.4.5 Study Limitations

There were several limitations that we noted about the data used in the current meta-analysis. The amount of heterogeneity in post-ablation AF comparison is worth noting. Although this was significant in some comparisons, we believe that the level of heterogeneity was not critical and may not have affected our risk estimates. The use of serum Gal-3 instead of local atrial Gal-3 may limit the clinical application of our results. It is crucial to note that serum Gal-3 changes may underlie systemic fibrotic diseases not just AF.

2.5 CONCLUSIONS

The present meta-analysis demonstrates an independent association between galectin-3 and AF. Our findings show that serum Gal-3 is increased in prevalent AF compared to no pre-existing AF. High Gal-3 levels associate with 57% greater risks of incident AF compared to low biomarker levels. More crucially, 1-SD increment in Gal-3 levels is predicts 44% greater risks of incident AF, persisting even persisting after correcting for baseline AF risk factors and comorbidities. Furthermore, Gal-3 is associated with AF severity, with serum Gal-3 more greatly increased in non-paroxysmal AF than in paroxysmal AF. In contrast to these findings, baseline Gal-3 levels do associate AF recurrence post-CA ablation, such that the association with AF is lost after pooling multivariate adjusted risk estimates.

2.6 TABLES

Table 1. Clinical Characteristics of Studies Reporting Galectin-3 and Atrial Fibrillation

Study ID	Year	Country	Design	Follow-up (year)	Quality Score	Mean Age (y)	Participants (% male)	Unit of Gal-3	AF Cases (%)
Clementy et al	2014	France	Cross-sectional	N/A	4	62.0±10.0	187 (68)	ng/ml	187 (100)
Gurses et al	2015	Turkey	Observational, case-control	N/A	6	N/A	151(47)	ng/ml	76 (50)
Wu et al	2015	China	Prospective, Community-based	N/A	6	N/A	96 (96)	ng/ml	50 (52)
Kornej et al	2015	Germany	Case-control	N/A	5	N/A	119 (63)	ng/ml	105 (88)
Sonmez et al	2015	Turkey	Case-control	N/A	4	71±8	85 (38)	pg/ml	52 (61.2)
Pavlovic et al	2017	Serbia	Case-cohort	1.25	6	68.1±10.9	54 (59.3)	ng/ml	32 (59.3)
Selcoki et al	2016	Turkey	Case-control	N/A	6	N/A	84 (44)	ng/ml	46 (54.8)
Stanojevic et al	2019	Serbia	Case-control	N/A	7	66.3±11.3	88 (36.4)	ng/ml	51 (57.9)
Begg et al	2017	UK	Case-control	N/A	6	N/A	129 (69)	ng/ml	92 (71.3)
Ho et al	2014	USA	Prospective cohort	11.2	8	59.0	3306 (47)	ng/ml	250 (7.5)
Fashanu et al	2017	USA	Prospective, epidemiological	15.7	8	62.6±5.6	8436 (41.3)	ng/ml	1185 (14.05)
Total					**6±1.3**		**12,735 (44.2)**		**2126 (33.5)**

Table 2. Characteristics of Randomised Clinical Trials

Study ID	Year	Country	Design	Follow-up (year)	Risk of Bias	Mean Age (y)	Participant (% male)	Gal-3 Unit	AF Cases (%)
PROTECT Study	2013	USA	Single-centre, prospective, RCT	0.8±0.2	Moderate	N/A	151 (84)	ng/mL	61 (40.4)
TME-CHF Study	2016	Switzerland & Germany	Multicentre, multinational, RCT	1.3±0.5	Moderate	78±7	219 (64)	ng/mL	61 (28)
GISSI-HF Study	2016	Italy	Multicentre, RCT	3.9±1.3	Low	66±11	631 (89)	ng/mL	118 (18)
Total				**2.0 ±1.7**		**72±8.5**	**1001 (82.8)**		**240 (24)**

Table 3. Characteristics of Post-ablation AF Studies

Study ID	Year	Country	Design	Follow-up (year)	Quality Score	Mean Age (y)	Participants (% male)	Gal-3 Unit	AF Recurrence
Kornej et al	2015	Germany	Cohort	0.5	5	62±9	105 (63)	ng/ml	36 (39.1)
Clementy	2016	France	Cohort	1.0	6	61±10	160 (71)	ng/ml	55 (34.4)
Wu et al	2015	China	Prospective, Community-based	1.42±0.34	7	48.9±7.8	96 (96)	ng/ml	32 (64)
Begg et al	2018	UK	Prospective cohort	1.0	4	N/A	92 (69.6)	ng/ml	42 (45.6)
Total				**0.98±0.4**	**5.5±1.3**	**57.3±7.3**	**453 (74.2)**		**165 (36.4)**

2.7 FIGURE LEGENDS

Figure 1. CONSORT Diagram of the Search Strategy

Figure 2. Risk of Bias Chart: A Summary of Judgements of 'Risk of Bias' in the Included Randomised Clinical Trials

Figure 3. Galectin-3 and Prevalent AF

Figure 4. Evaluation of Association of Galectin-3 and AF Incidence in Randomized Clinical Trials

Figure 5. Evaluation of Galectin-3 and Risk of Incident AF in Cohort Studies

Figure 6. Galectin-3 and AF Severity

Figure 7. Galectin-3 and Post-ablation AF

Figure 8. Galectin-3 and Risk of Post-ablation AF

Figure 1. CONSORT Diagram of the Search Strategy

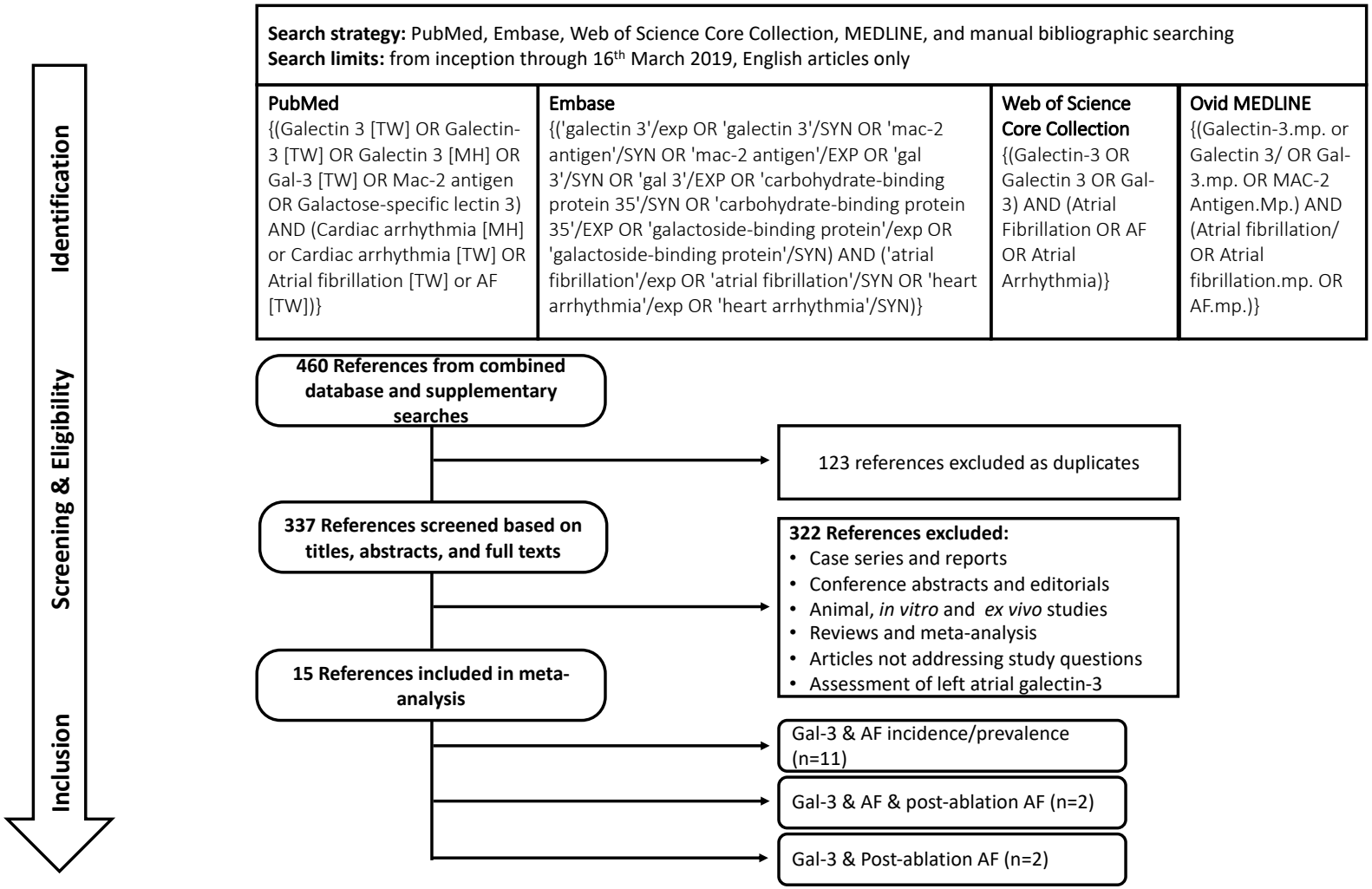


Figure 2. Risk of Bias Chart: A Summary of Judgements of 'Risk of Bias' in the Included Randomised Clinical Trials

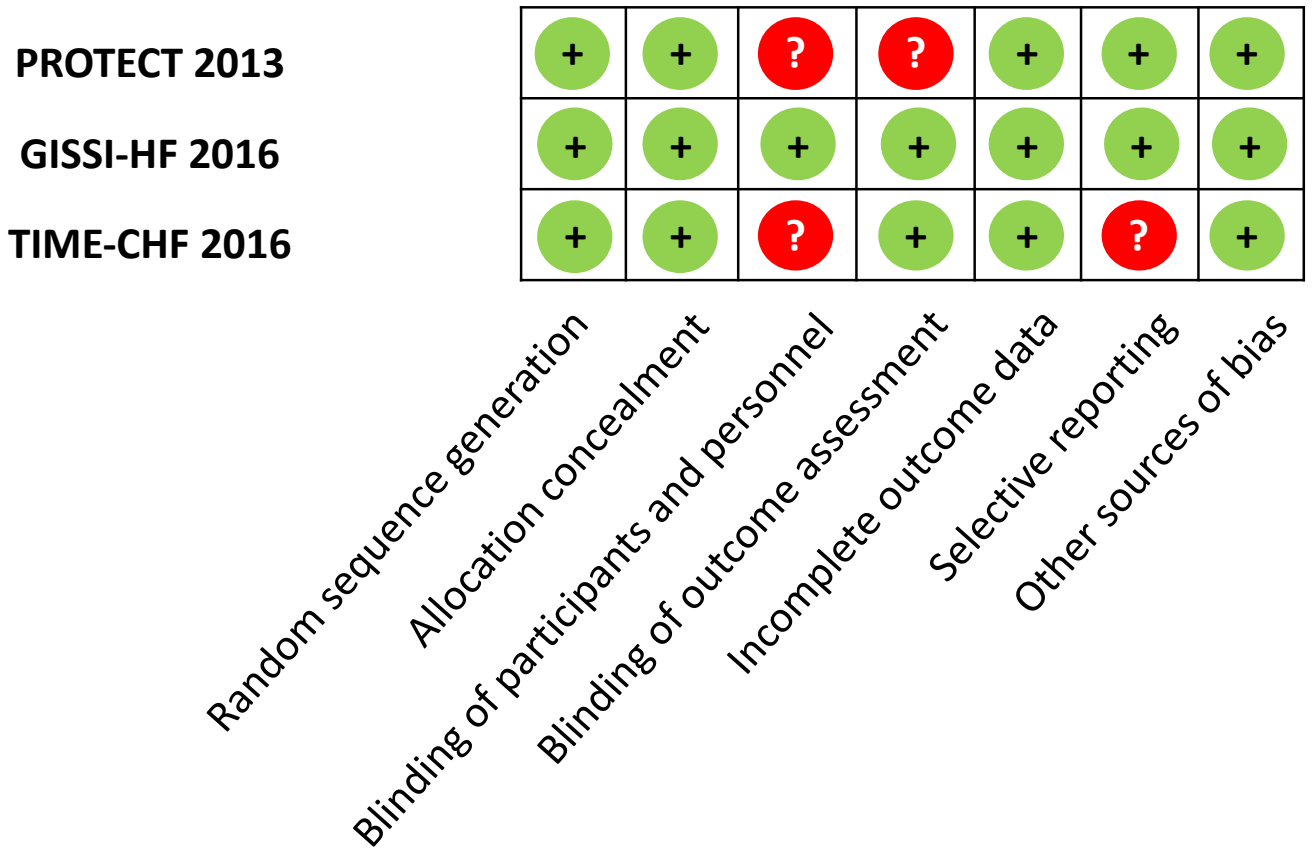


Figure 3. Galectin-3 and Prevalent AF

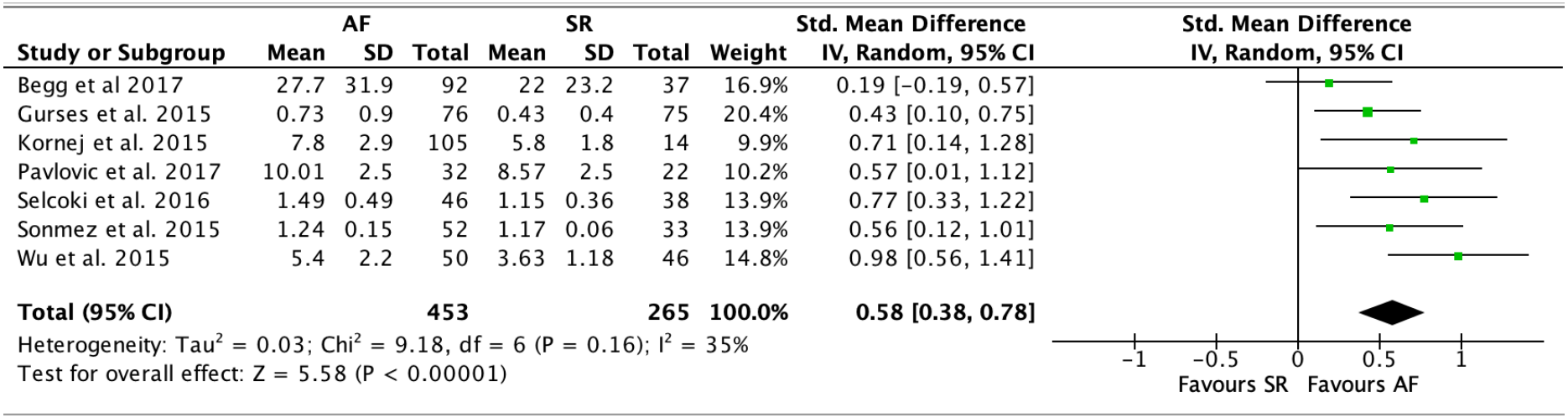


Figure 4. Evaluation of Association of Galectin-3 and AF Incidence in Randomized Clinical Trials

Study or Subgroup	High Gal-3 Events	High Gal-3 Total	Low Gal-3 Events	Low Gal-3 Total	Weight	Risk Ratio M-H, Random, 95% CI
GISSI-HF 2016	35	151	81	480	40.9%	1.37 [0.97, 1.95]
PROTECT 2013	35	75	26	76	32.5%	1.36 [0.92, 2.02]
TIME-CHF 2016	36	110	25	109	26.6%	1.43 [0.92, 2.21]
Total (95% CI)		**336**		**665**	**100.0%**	**1.38 [1.11, 1.73]**
Total events	106		132			

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.03$, df = 2 (P = 0.99); $I^2 = 0\%$

Test for overall effect: Z = 2.83 (P = 0.005)

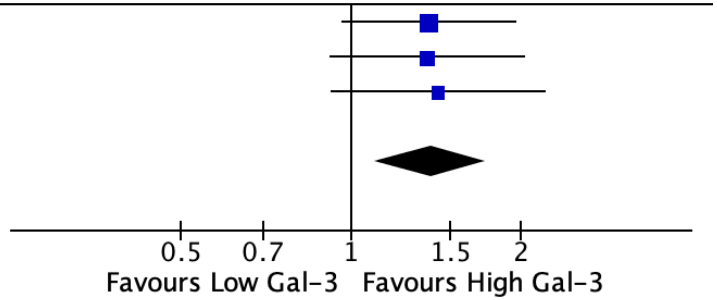


Figure 5. Evaluation of Galectin-3 and Risk of Incident AF in Cohort Studies

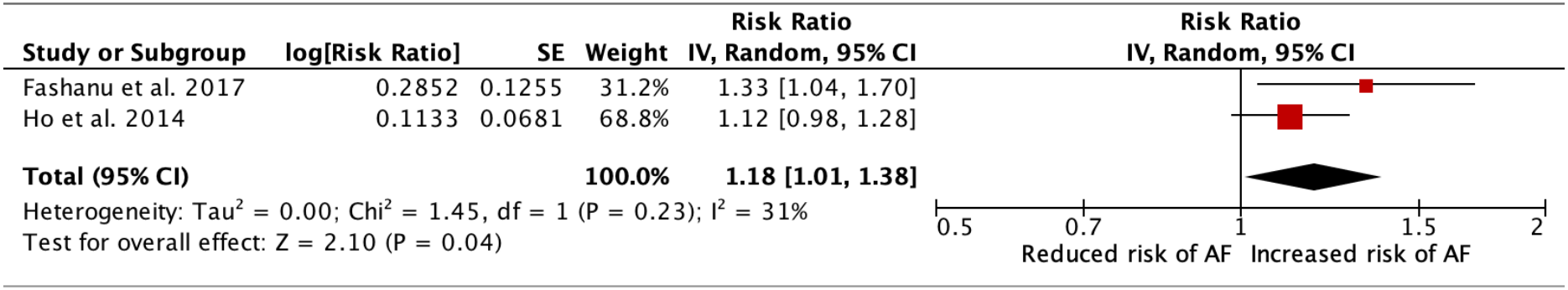


Figure 6. Galectin-3 and AF Severity

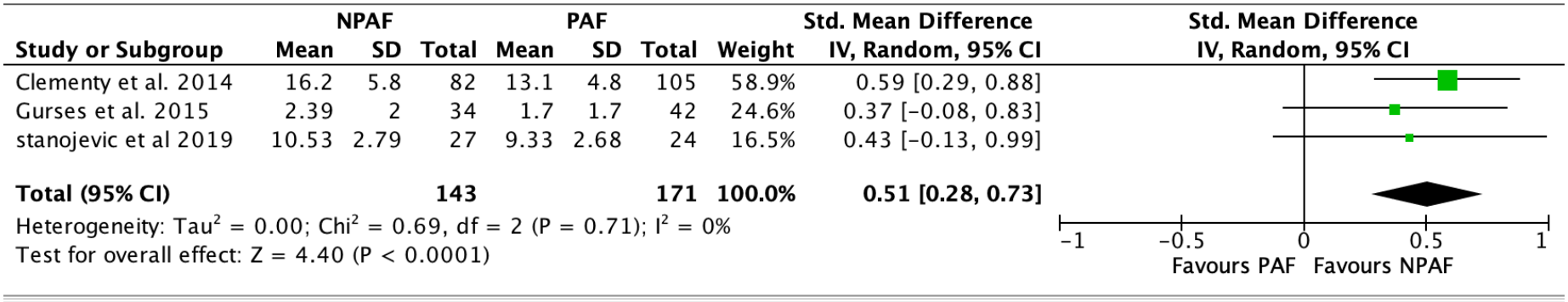


Figure 7. Galectin-3 and Post-ablation AF

Study or Subgroup	Recurrent AF Mean	SD	Total	No AF Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Begg et al 2018	30.8	55.8	42	24.5	29.6	50	24.6%	0.14 [-0.27, 0.55]
Clementy et al. 2016	16	6.6	55	13.5	4.8	105	26.4%	0.45 [0.12, 0.78]
Kornej et al. 2015	7.08	2.7	36	8.09	3.2	83	25.0%	-0.33 [-0.72, 0.07]
Wu et al. 2015	6.03	2.12	32	4.28	2.03	64	23.9%	0.84 [0.40, 1.28]
Total (95% CI)			**165**			**302**	**100.0%**	**0.27 [-0.19, 0.74]**

Heterogeneity: $Tau^2 = 0.18$; $Chi^2 = 16.90$, df = 3 (P = 0.0007); $I^2 = 82\%$

Test for overall effect: Z = 1.16 (P = 0.25)

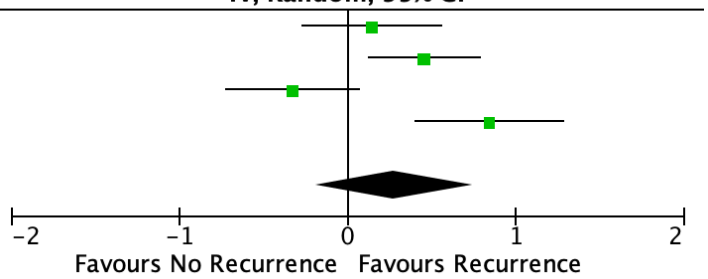
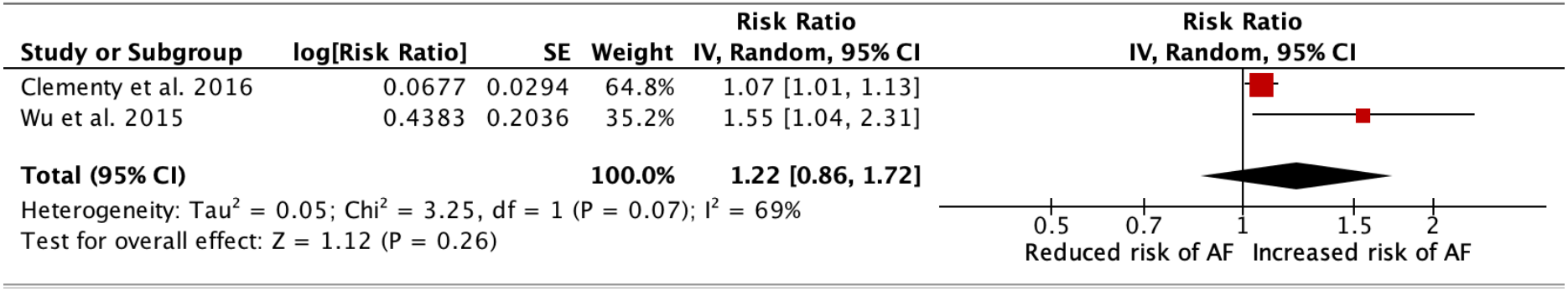


Figure 8. Galectin-3 and Risk of Post-ablation AF



3. Chapter Three

Epicardial Adipose Tissue and Atrial Fibrillation Risk: A Systematic Review and Meta-Analysis

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3.1 INTRODUCTION

A growing body of evidence has demonstrated atrial fibrillation (AF) as a cardiovascular epidemic, which is associated with reduced quality of life and increased risk of stroke and heart failure.[12, 13, 29] Moreover, new-onset AF occurring after open heart surgery (post-operative AF, POAF) is recognised as a significant complication during the post-operative period, affecting 20% to 60% of post-surgery patients.[381-385] It is shown to be associated with long-term complications, such as worsening of cardiac haemodynamics, increased incidence of ventricular arrhythmias, heart failure, cognitive impairment, and increased mortality.[22, 386, 387]

The burgeoning burden of AF has been attributed to the emergence of novel risk factors, such as obesity. Indeed, obesity accounts for 20% of all AF and 60% of the rising incidence rate of the arrhythmia.[22] More recently, epicardial adipose tissue (EAT) has emerged as an important element in the pro-arrhythmic substrate formation, with reports showing that it might explain the clinical link between obesity and AF.[263, 265, 266, 300] EAT is a metabolically active fat depot found on the visceral layer of the pericardium and in close proximity to the myocardium, sharing the same microcirculation with the cardiac musculature.[182, 263] This unique anatomic position has raised the postulate of a paracrine effect on cardiac musculature. Accordingly, EAT expansion has been reported in experimental models[181, 182] and shown to underlie increased secretion of pro-fibrotic and inflammatory cytokines.[278] Indeed, EAT has been associated with induction of atrial interstitial fibrosis (the histological surrogate for atrial structural remodelling) and fat cell infiltration in animal models, and increased inflammatory activity in patients with AF.[182, 280, 388]

In this study, we hypothesised that expansion of the ectopic fat pad might predispose individuals to excess risk of developing AF, increased risk of relapse following catheter ablation and increased incidence of AF post-surgery. Therefore, the aims of this systematic review of the literature and meta-analysis were to evaluate the association between EAT and (1) AF prevalence and incidence; (2) severity of AF (non-paroxysmal vs. paroxysmal forms); (3) recurrence of AF post-ablation; and (4) incidence of AF post-cardiac surgery.

3.2 METHODS

3.2.1 Literature Search Strategy

This meta-analysis was registered on **PROSPERO (ID: CRD42018105707)** and conducted according to the guidelines given by the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) and Meta-analysis of Observational Studies in Epidemiology (MOOSE). We identified the studies used for this systematic review and meta-analysis through online database search done on PUBMED, EMBASE, Ovid MEDLINE and the Core Collection of Web of Science. Full keywords used are as follows:

PubMed: (Fat [TW] OR Adipose Tissue [TW] OR Adipose Tissue [MH] OR Adipocyte* [TW] OR Adipocyte* [MH]) AND (Epicardi* [TW] OR Epicardi* [MH] OR Pericardi* [TW] OR Pericardi* [MH] AND (Cardiac Arrhythmia [MH] OR Cardiac Arrhythmia [TW] OR Atrial Fibrillation [TW] or AF [TW])

EMBASE: ('Fat'/EXP OR ‘Fat’/SYN OR 'Adipocyte'/EXP OR ‘Adipocyte’/SYN OR 'Adipose Tissue'/EXP OR 'Adipose Tissue'/SYN) AND (‘Epicardi*’/EXP OR ‘Epicardi*’/SYN OR ‘Pericardi*’/EXP OR ‘Pericardi*’/SYN) AND ('Atrial Fibrillation'/EXP

OR 'Atrial Fibrillation'/SYN OR 'Heart Atrium Arrhythmia'/EXP OR 'Heart Atrium Arrhythmia'/SYN OR AF)

The Core Collection of Web of Science: ("Adipose Tissue" OR Fat OR Adipocyte* OR Lipocyte*) AND (Pericardi* OR Epicardi*) AND ("Atrial Fibrillation" OR "Supraventricular Arrhythmi*" OR AF OR "Atrial Arrhythm*")

Ovid MEDLINE: (Adipocyte.mp. or Adipocytes/ or Fat.mp. OR Adipose Tissue.mp.) AND (Epicardi*.mp. OR Pericardium/ OR Pericardi*.mp.) AND (Atrial Fibrillation/ OR AF.mp. OR Arrhythmias, Cardiac/)

We conducted online database searches from inception through to 5 July 2018, with retrieved papers exported to and sorted by EndNote X8.2 software.

3.2.2 Inclusion and Exclusion Criteria

Screaming for eligibility and inclusion was conducted by two investigators, with any disagreement settled by consensus. We screened the retrieved papers based upon the titles followed by the scrutiny of their abstracts and full-texts to ensure nothing was missed. Papers were first excluded based on the following criteria: (1) publication in non-English languages; (2) whether they were conference reports and abstracts that were not yet published; (3) editorials and letters to the editor; (4) case reports. The reference lists of review articles were searched for relevant original papers and excluded thereafter. In the next stage, the full-texts of the references were screened, with non-relevant studies excluded thereafter. Finally, we included studies if they reported on: (1) the association between EAT/pericardial fat and the prevalence or incidence of AF; (2) EAT and outcome after catheter ablation of AF; (3) association of EAT with non-paroxysmal (Non-PAF) AF vs. PAF; (4) the association

between the EAT and post-operative AF; and (5) maintenance or dissection of the anterior epicardial fat pad and post-op AF.

3.2.3 Study Selection and Data Extraction

The study selection and data extraction were done by two investigators (Thomas Agbaedeng and Andien Munawar), using an a priori determined set of guidelines with any disagreement resolved by consensus or with a third author, where necessary. The following outcomes and data were collected: (1) AF incidence, prevalence, recurrence after catheter ablation; (2) EAT/pericardial fat thickness and volume; (3) Risk estimates; (4) Study endpoints; (5) Study design; (6) Participants; (7) EAT measurement modality; (8) study country; and (9) imaging modality.

3.2.4 Risk of Bias and Quality Assessment

The methodological qualities of the included studies were assessed on the bases of the study design using the Newcastle-Ottawa Scale (NOS) for assessing the quality of non-randomised studies in meta-analyses. The scales for case-control studies and cohort studies were used to assess the quality of case-control and cohort studies, respectively. The following perspectives were used to evaluate quality of cohort studies:

1. Selection of study groups,
2. comparability of these study groups, and
3. The ascertainment of the outcome of interest.

And the following for case-control studies:

1. The selection study groups,

2. The comparability of the participant groups, and
3. The ascertainment of the exposure of interest.

The judgement of the studies was done using a "Star System" and computed as quality scores ranging from 1 to 9. A quality score of 1 indicated an extremely poor methodological design and a score of 9 was indicative of a very good quality.

Due to the apparent limitation of quality checklists and scales for intervention studies, we further assessed ablation studies using "a Cochrane Risk of Bias Assessment Tool: for Non-Randomised Studies of Intervention (ACROBAT-NRSI)." We assessed the risk of bias (RoB) in observational studies based on seven domains through which bias is likely to be introduced into these studies, namely:

1. Bias due to confounding,
2. Bias in selection of participants into the study,
3. Bias in measurement of interventions,
4. Bias due to departures from intended interventions,
5. Bias due to missing data,
6. Bias in measurement of outcomes, and
7. Bias in selection of the reported result.

See **Table 1** for the criteria used.

3.2.5 Data Synthesis and Analysis

A random effects meta-analysis was conducted on the pooled results from the various citations using RevMan (The Cochrane Collaboration, Copenhagen). Two meta-analytic effects size types were used for the data analyses, namely: standardised mean difference

(SMD) and odds ratios (OR). We used SMD to present the change or difference in EAT on a uniform scale, insofar as the studies reported heterogenous definitions of EAT (total epicardial, peri-atrial, and/or pericardial) measurement. SMD typically measures the size of an outcome relative to the standard deviation (SD) of the outcome, thus, reflecting the real differences in the variability of the measured outcomes. OR's were pooled from studies that conducted multivariable analysis, and this was done to show independent association with AF as well as a prediction of AF.

EAT was pooled as a volumetric measure or thickness measure and presented as mean and standard deviation (mean±SD). Where EAT was reported as median and interquartile ranges (IQR), we converted these to mean±SD using models derived by Wan et al[389] The degree of heterogeneity in outcomes across the studies was assessed by examination of forest plots, chi-squared (Chi^2) test and I-squared (I^2) statistic. The latter two provide numerical values for an assessment of heterogeneity, with a high Chi^2 relative to the degree freedom suggestive of variations in effect estimates and I^2 greater than 50% indicative of a considerable amount of heterogeneity ($p<0.05$ defined as the cut-off). Statistical significance was set at $p<0.05$

3.3 RESULTS

3.3.1 Search Result and Synthesis of the Literature

The online database searches and supplementary searches conducted resulted in a total of 1316 references. We excluded 1257 articles that did not meet our inclusion criteria. Thirty-three observational studies with a total of 24,091 participants (59.1% males and 40.9% females) were included in this review. Analysis was done for: (1) epicardial fat and prevalent

AF; (2) epicardial fat and incident AF from cohort studies; (3) epicardial fat and recurrent AF after catheter ablation; and (4) epicardial fat and POAF after cardiac surgery. A full description of the characteristics of the included studies, including study designs, quality scores, demographics, methodology, study endpoints, follow-up, and participants, is provided in **Tables 2 through 5**. An overview of the search strategy and selection methodology is shown as a flowchart in **Figure 1.**

3.3.2 Epicardial Fat and Prevalent AF

Eighteen studies[264, 265, 270, 303, 305, 390-397] investigated the prevalence of AF, corresponding to 7,738 participants (46.8% females). Computed tomography (CT) was the predominant technique (75%) for EAT measurement, followed by transthoracic echocardiography (TTE) (20%), and cardiac magnetic resonance (CMR) (5%), see **Table 2**. Overall, we found significantly increased EAT volume in patients with AF as compared to sinus rhythm, with a SMD of 0.72 (95% Confidence Interval, CI: 0.49 to 0.95; p<0.001) (**Figure 2**). However, there was significant heterogeneity in this comparison (I^2 >84%; p<0.001). Subgroup analysis showed that the heterogeneity was contributed by Greif et al[393], which measured pericoronary EAT from coronary calcium score CT images. We next excluded Greif et al[393] and still found significant difference in EAT between AF and SR controls ([SMD: 0.79; 95% CI: 0.64 to 0.93; p<0.001] and [I^2; p=0.12]). When we pooled the multivariable-adjusted OR's from seven studies reporting this, 1-standard deviation (SD) increase in EAT volume was significantly and independently associated with the presence of AF (OR: 1.03; 95% CI: 1.00 to 1.05; p=0.03), **Figure 3**. This comparison had substantial heterogeneity ([I^2: 79%; p<0.001], **Figure 3**), which was lost after limiting the analysis to Kanazawa et al[303],

Mahabadi et al[394] and Sevinc et al[395] (I^2: 49%; p=0.14). In a sub-analysis involving only left atrial EAT, significant association with AF presence was also noted ([SMD: 0.78; 95% CI: 0.43 to 1.12; p<0.001], **Figure 4**). The heterogeneity in this analysis was moderate and not significant (p=0.08; **Figure 4**). Similarly, EAT was significantly thicker in prevalent AF patients as compared with controls (SMD: 1.30; 95% CI: 0.36 to 2.24; p=0.007), **Figure 5**. But there was considerable heterogeneity ([I^2=97%, p<0.001], **Figure 5**), which was contributed by Yorgun et al,[396] ([SMD: 1.70, 95% CI: 1.13 to 2.28, p<0.001] and [I^2: 80%, p=0.02] after removing source of heterogeneity).

3.3.3 Epicardial Fat and Incident AF

Three cohort studies[394, 398, 399] reporting incident AF, corresponding to 14,031 individuals (54.2% females) with EAT measurement performed using CT, **Table 3**. Patients were followed up for a mean of 7.3 years (SD: 2.4) and 8.8% of them (968) had incidence of new-onset AF. In the pooled analysis, there was no significant association between EAT volume and the risk of incident AF, (OR: 1.07; 95% CI: 0.99 to 1.15; p=0.10), see **Figure 6**. The three studies were well matched, with no statistical evidence of heterogeneity (I^2=0%; p=0.95).

3.3.4 Epicardial Fat and Severity of AF

The relationship between epicardial fat and the severity of AF was reported in 14 studies[265, 270, 302, 303, 306, 390-393, 400-404] (2,533 patients; 30% females). From the pooled analysis, both total and left atrial EAT volumes were significantly increased in the patients with non-paroxysmal AF compared to those with paroxysmal AF (total EAT [SMD: 0.46, 95% CI: 0.24 to 0.67,

p<0.001]; LA-EAT [SMD: 0.62, 95% CI: 0.26 to 0.98, p<0.001], see **Figures 7 & 8**). Pooled analysis of EAT thickness showed similar results, with non-PAF patients having 1.34 SMD greater EAT than PAF patients (SMD: 1.34; 95% CI: 0.65 to 2.02; p<0.001), **Figure 9**. Pooled analyses involving total EAT volume and LA-EAT volume had moderate amounts of heterogeneity (total EAT [total EAT [I^2: 69%, p<0.001] and [I^2: 54%; p=0.05)], **Figures 7 & 8**). In total EAT, heterogeneity was caused by inclusions of Kim et al[402], Masuda et al[403] and Nakamori et al[404] ([SMD: 0.60, 95% CI: 0.44 to 0.77, p<0.001] and [I^2: 20%; p=0.27] after exclusions); and by Nakamori et al[404] in LA-EAT ([SMD: 0.72, 95% CI: 0.38 to 1.07, p<0.001] and [I^2: 43%, p=0.14]). Furthermore, considerable heterogeneity was noted for EAT thickness ([I^2: 88%, p<0.001], **Figures 9**) and was contributed by Iacobellis et al[401] ([SMD: 1.68, 95% CI: 1.40 to 1.96, p<0.001] and [I^2: 17%, p=0.27] after exclusion).

3.3.5 Epicardial Fat and Recurrent AF

Ten cohort studies[263, 390, 392, 397, 400, 402, 403, 405-407] reported on AF recurrence after catheter ablation with a total of 1,938 patients (31.6% females) (**Table 4**). After a mean follow-up period of 18.6±4.6 months, 523 (27.0%) AF patients undergoing catheter ablation experienced AF recurrence, defined as AF or atrial tachycardia >30 seconds after 3-month blanking periods. The dominant technique for estimating EAT was CT (70.0%), with only three studies using CMR and TTE. Total EAT volume was significantly increased in the recurrent AF group ([SMD: 0.49; 95% CI: 0.01 to 0.98; p=0.05], **Figure 10**), with independent association demonstrated upon multi-variable adjustment, **Figure 11**. In three studies where EAT thickness was assessed, there was also significant correlation between EAT and recurrent AF, ([SMD: 0.98; 95% CI: 0.64 to 1.93; p<0.001], **Figure 12**). However,

there was evidence of significant heterogeneity amongst some of the studies ([I^2: 75%, p<0.007 & 0.02], **Figures 10 & 12**). The source of heterogeneity was traced to EAT depots assessed in Nagashima et al[390] and Nakatani et al[406] (I^2: 53%, p=0.14), and CT assessment of EAT thickness in Kocyigit et al[405] (I^2: 51%, p=0.15).

3.3.6 Epicardial Fat and Incident AF Post-cardiac Surgery

Two studies[408, 409] investigated the effects of total epicardial fat on the incidence of post-operative AF and involved a total of 185 participants (24.9% females). All were prospective cohort studies; Opolski et al[409] had a retrospective design, whereas Drossos et al[408] had a prospective design, respectively, **Table 5**.The results of the meta-analysis showed that epicardial fat was significantly increased in patients with incident post-operative AF compared to individuals in sinus rhythm (SMD: 0.87; 95% CI: 0.34 to 1.39; p=0.001), see **Figure 7**. The amount of heterogeneity in the analysis was moderate and did not reach statistical significance (I^2 = 59%; Chi^2 = 4.93; p=0.08).

3.3.7 Assessment of Risk of Bias

The methodological study quality was assessed using the NOS quality scale, running on a 1-9 scale. Generally, the quality was good across these studies, and ranged from moderate to high quality ([mean±SD: 6.4±0.9, 8.0±1.0, and 6.3±1.2 for prevalent, incident and recurrent AF, respectively] **Tables 2 through 5**). Further, risk of bias assessments were performed for all included studies and summarised in **Figure 8.**

3.4 DISCUSSION

3.4.1 Major Findings

Epicardial fat has been implicated in the risk of cardiovascular disease and is currently being investigated as the mechanistic link between increasing adiposity and the development of AF. In this meta-analysis, we sought to thoroughly define the association that has been described between epicardial fat and AF. It demonstrates that:

1. Epicardial fat is significantly larger in patients with prevalent, but not associated with new-onset AF.
2. Increased epicardial fat, both as volumetric and thickness measures, is significantly associated with greater severity of AF.
3. The prognosis of AF after a radiofrequency catheter ablation is worsened with increasing amount of EAT. Increased EAT is significantly associated with the recurrence of AF after ablation.
4. Finally, epicardial fat is significantly elevated in patients with POAF.

3.4.2 Epicardial Fat and AF

EAT has emerged as an important visceral adipose tissue that may refine our understanding of the role of adiposity in AF risk, and has been implicated as the putative mechanistic link between obesity and AF. Batal et al[266], Al Chekakie et al[265], and Thanassoulis et al[264] independently reported an association between epicardial fat and AF prevalence less than a decade ago, and since then, EAT has been consistently shown to be larger in AF patients than in those in SR. Wong et al[263] associated total pericardial fat with 3.56 to 11.25 odds of AF

prevalence and chronicity, which was independent and much stronger than any traditional obesity marker, including BMI. In the same study, periventricular fat was associated with almost 4-fold increased risk of recurrent AF.[263] Interestingly, we have demonstrated that epicardial fat is significantly larger in patients with prevalent and new-onset AF, with 1-unit increment in EAT volume associated with more than excess odds of AF occurrence independently of traditional risk factors. Our pooled analyses also showed associations of EAT with progression of AF after catheter ablation.

3.4.3 AF Substrate Due to Epicardial Fat

EAT is a unique and metabolically active fat depot subtending the visceral layer of pericardium and in close proximity to the myocardium, a feature that has ignited much interest in its potential paracrine effects on cardiac musculature.[182, 278, 280] Accordingly, in clinical studies, EAT has been significantly associated with marked conduction abnormalities[300], electrical imbalance[283], cardiac autonomics dysfunction[290], and increased inflammatory activity[271].

The pro-arrhythmic mechanism of expanding EAT is probably complex. We previously demonstrated expansion of epicardial fat with progressive weight gain and obesity, with a consequent induction of fat cell infiltration of the posterior left atrial myocardium.[182] The secretome of EAT has been reported to induce global fibrosis of rat atria[278], the histological hallmark of structural remodelling, with fibrotic remodelling of EAT significant correlated with atrial interstitial fibrosis in left atrial appendage samples from humans[388]. We hypothesised that fibro-fatty infiltrations are sufficient to create AF substrate, which might be driven by loss of cell-cell coupling, increased local conduction blocks and

conduction heterogeneities.[131] Additionally, co-incubation with EAT adipocytes is shown to cause abnormal remodelling of membrane currents, afterdepolarisations and increased ectopic activities in experimental models, further highlighting the important role of infiltrating EAT adipocytes in formation of AF substrate.[297]

3.4.4 Post-operative AF Substrate Due to Epicardial Fat

POAF, like other clinical forms of AF, has a complex pathophysiological mechanism and several loops have been implicated, such as local and systemic inflammation, oxidative stress, neurohumoral cascade activations, and ion channel remodelling. Intriguingly, there is an increasing body of evidence to suggest that EAT may contribute to the atrial arrhythmogenic substrate, which can predispose to and maintain POAF. In 76 patients undergoing CABG, Viviano et al[276] demonstrated that gelsolin, an anti-inflammatory protein, in the secretome of epicardial fat was predictive of maintenance of sinus rhythm post-surgery. Noteworthy, by reducing expression of anti-inflammatory factors like gelsolin and directly inducing pro-inflammatory cytokine production, as seen in the peri-operative settings[271, 272, 388, 410], EAT could heighten the vulnerability of atrial tissue to inflammatory cascades that ultimately leads to formation of POAF substrate. More importantly, the presence of an established AF substrate, such as fibro-fatty infiltrates, directly relates to EAT and might impact the POAF risk. Indeed, the degree of fibrosis and P-wave duration during the start of open-heart surgery was shown to be predictive of POAF.[411]

3.4.5 Limitations

There are a few limitations that are worth noting regarding the data in the current meta-analysis. We found a significant amount of heterogeneity in a number of our pooled analyses. Additionally, the use of overwhelmingly non-randomised observational studies as opposed to randomised controlled trials is an important drawback and may have biased our analysis. Notwithstanding this limitation, the methodological quality of these studies was good, ranging from moderate to high quality scores, as well as their risk of bias level.

Finally, the definition of epicardial fat varied in the included studies, with some reporting it as 'epicardial adipose tissue' and others as 'pericardial fat'; while others use both definitions interchangeably. This is partly to do with the inconsistency in the literature regarding what constitute epicardial adipose tissue and pericardial adipose tissue.[412] EAT pertains to the fat lying contiguously with the myocardium and found between the latter and the visceral layer of the pericardium.[413] Pericardial fat is a loose term because it incorporates all the fat found around the heart, including the EAT and another adipose tissue 'paracardial fat', which is located externally to the parietal pericardial layer or membrane.[413] One important drawback to this vague distinction of these fat zones is that it may lead to overestimation or underestimation of the reported values of EAT.

3.4.6 Clinical Implications

These findings reinforce the clinical associations reported between expansion of epicardial fat and AF. Although the best imaging technique to quantify and characterize EAT amount and distribution remains unclear, EAT may represent an interesting risk marker to identify patients with increased AF risk which could allow a more personalized risk stratification.

EAT may hold promise as a novel target for atrial fibrillation, a concept recently tested in patients who underwent pulmonary vein isolation wherein treatment with atorvastatin led to reduction in EAT volume.[414] In addition to pharmacological interventions, combined modification of risk factors, which are individually associated with EAT, and weight loss may be another strategy to target EAT.[345, 415] Further studies are warranted to improve our understanding of EAT-mediated atrial remodelling and to determine whether its reduction constitutes a treatment target for primary and secondary prevention of AF.

3.5 CONCLUSIONS

This meta-analysis provides evidence for an independent association between EAT and AF. We show that EAT is significantly increased in patients with prevalent AF, and that this association is independent of traditional risk factors. EAT was not associated with incident new-onset AF, but significantly associated with greater severity of AF, and recurrence of AF after catheter ablation. Moreover, increased amount of EAT was also significantly correlated with development of POAF.

3.6 TABLES

Table 1. Criteria for assigning risk of bias to studies based on the Cochrane guideline

RESPONSE OPTION	CRITERIA
Low risk of bias	The study *MUST* be judged to be at low risk of bias for all domains.
Moderate risk of bias	The study *MUST* be judged to be at low or moderate risk of bias for all domains (i.e., there is moderate risk of bias in at least one domain).
Serious risk of bias	The study *MUST* be judged to be at serious risk of bias in at least one domain, but not at critical risk of bias in any domain.
Critical risk of bias	The study *MUST* be judged to be at critical risk of bias in at least one domain.
No information on which to base a judgement about risk of bias.	There is no clear indication that the study is at serious or critical risk of bias and there is a lack of information in one or more key domains of bias.

Table 2. Summary characteristics of studies investigating epicardial fat and prevalent AF

Study ID	Country	Design	Study quality	Participants (% males)	EAT measure (technique)	AF diagnosis	Type of AF (% PAF)
Muhib et al. 2013	Japan	Case-Control, retrospective	6	62 (58)	Area (MRI)	ECG/Holter/medical records	PAF (100)
Greif et al. 2013	Germany	Retrospective	6	1,288 (58.9)	Volume (CT)	Interview/ECG/medical history	PAF & PerAF (63)
Al Chekakie et al. 2010	United States	Case-Control	6	273 (50.2)	Volume (CT)	NS	PAF & PerAF (64)
Shin et al. 2011	South Korea	Retrospective	5	80 (72.5)	Volume (CT)	NS	PAF & PerAF (50)
Kanazawa et al. 2014	Japan	Case-Control	5	240 (79.6)	Volume (CT)	NS	PAF & PerAF (66.7)
Batal et al. 2010	United States	Case-Control	7	169 (65.1)	Thickness (CT)	NS	PAF PerAF (62.5)
Nagashima et al. 2011	Japan	Case-Control	5	77 (76.6)	Volume (CT)	12-L ECG & 24-h holter	PAF & PerAF (60)
Nagashima et al. 2012	Japan	Not specified	N/A	34 (85)	Volume (CT)	12-L ECG/medical history/physical exam	PAF & PerAF (47)
Acet et al. 2014	Turkey	Case-control	6	197 (46.7)	Thickness (TTE)	–	Non-valvular (53)
Yorgun et al. 2015	Turkey	Retrospective	7	618 (53.2)	Thickness (CT)	Resting ECG; 24-h Holter; interview; & medical records	Non-valvular (39.7)

Table 2. CONT…

Study ID	Country	Design	Study quality	Participants (% males)	Eat measure (technique)	AF diagnosis	Type of AF (% PAF)
Tsao et al. 2011	Taiwan	Case-Control	6	102 (71.6)	EAT volume (CT)	NS	Not specified (63.2)
Iacobellis et al. 2014	United States	Cross-Sectional	5	84	EAT thickness (TTE)	ECG or 24-h Holter	PeAF | PAF (23.8)
Masuda et al. 2015	Japan	Cross-sectional	6	53 (68)	LA-EAT | total EAT volume (CT)	NS	PAF | PerAF (42)
Sevinc et al. 2017	Turkey	Retrospective, case-control	7	132 (37.1)	Atrial pericardial fat (CT)	NS	PerAF (0)
Girerd et al. 2013	France	Cross-sectional	6	49 (83.7)	EAT volume (CT)	NS	PAF | PeAF (51)
Akdag et al. 2015	Turkey	Cross-sectional	6	148 (61.5)	EAT thickness (TTE)	ECG or cardiologist-assessed	NS
Mahabadi et al. 2014	Germany	Prospective cohort	8	3905 (47)	EAT volume (CT)	12-L ECG	NS
Nakamori et al. 2018	United States	Case-control	7	105 (64)	LA-EAT volume (CT)	NS	PAF | PerAF | LS-PerAF (74)
Subtotal			**6.4±0.9**	**7,738 (53.4)**			

Table 3. Summary of cohort studies investigating epicardial fat and AF

Study ID	Country	Design	Study quality	Follow-up (year)	Participants (% male)	AF diagnosis	Incidence of AF (%)	EAT measure (technique)
Mahabadi et al. 2014	Germany	Prospective cohort	8	5	3,905 (47)	12-L ECG	50 (1.4)	EAT volume (CT)
Lee et al. 2016	United States	Cohort	7	9.7	2,135 (46.7)	12-L ECG & holter	162 (7.6)	Pericardial fat volume (CT)
Heckbert et al. 2017	United States	Cohort	9	7.25	7991 (45)	ICD-9 code for AF or flutter	756 (9.5)	Pericardial fat volume (CT)
Total			**8±1**	**7.3±2.4**	**14,031 (45.8)**		**968 (8.8)**	

AF, atrial fibrillation; CA, catheter ablation; EAT, epicardial adipose tissue; ECG, electrocardiograph; CT; computed tomography; and ICD, international classification of disease code.

Table 4. Summary characteristics of studies evaluating epicardial fat and AF ablation outcome

Study ID	Country	Design	Duration	Participants	Study quality	Endpoint	AF diagnosis	Recurrence (% PAF)	EAT measure (technique)
Kim et al. 2014	South Korea	Cohort	19.3 ± 8.5	665 (76.7)	5	Sustained AF	ECG | 24/48-h Holter	176 (26.5)	Pericardial fat volume (CT)
Wong et al. 2011	Australia	Cross-sectional	21.0 ± 12	122 (76)	7	Recurrence	7-d ambulatory cardiac monitoring	12 (11.8)	Atrial/ventricular/total pericardial fat volume (CMR)
Nagashima et al. 2011	Japan	Case-control	10.2	40 (77.5)	6	Recurrence ≥2 months post-CA	12-lead ECG & Holter	15 (37.5)	Left-atrial & total EAT volume (CT)
Tsao et al. 2011	Taiwan	Case-control	7.5 ± 2.6	68 (76.5)	7	AT recurrence or repeat CA	24-hr Holter	24 (35.3)	Atrial EAT volume (CT)
Chao et al. 2013	Taiwan	Case-control	16±9	283 (69.6)	6	NS	24-h Holter	95 (33.6)	EAT thickness (TTE)
Canapolat et al. 2016	Turkey	Prospective, cohort	19±8.1	234 (51.3)	6	NS	24-h Holter	45 (19.2)	EAT thickness (TTE)
Kocyigit et al. 2015	Turkey	Retrospective, cohort	28.3±29.6	249 (48.3)	6	AF recurrence	12-lead ECG or 24-h Holter	60 (24.1)	Atrial/ventricular/total EAT thickness (CT)
Stojanovska et al. 2015	United State	Retrospective, cohort	33.0±9.0	169 (76)	6	NS	Event monitor	45 (26.6)	EAT volume (CT)

Nakatani et al. 2015	Japan	Cohort	>12	55 (75)	5	AF >30s at >3 months post-CA	12-lead ECG & 24-h Holter	10 (18.2)	EAT volume (CT)
Masuda et al. 2015	Japan	Cohort	16±4.4	53 (68)	6			41 (77.4)	LA-/total EAT volume (CT)
Subtotal			**>18.6±4.6**	**1,938 (68.6)**	**6.0±0.7**			**482 (26.8)**	

AF, atrial fibrillation; CA, catheter ablation; CMR, cardiac magnetic resonance imaging; CT, computed tomography; EAT, epicardial adipose tissue; ECG, electrocardiograph; NS, not specified; PAF, paroxysmal AF; TTE, transthoracic echocardiography.

Table 5. Summary characteristics of studies evaluating the effects epicardial fact on post-operative AF

Study ID	Country	Design	Follow-up	Participants	Quality score	Intervention	Study endpoint	AF incidence	EAT measure (technique)
Drossos et al. 2012	Greece	Prospective cohort	Until discharge	83 (79.5)	7	Elective on-pump CABG	Any episode of POAF	23 (33.7)	Pericardial fat (CT)
Opolski et al. 2015	Poland	Retrospective, cohort	Until discharge	102 (75.5)	7	On- or off-pump CABG	New or recurrent AF	24 (23.5)	LA-EAT volume (CCTA)
Subtotal				**185 (75.1)**	**7±0**			**48 (25.4)**	

AF, atrial fibrillation; CABG, coronary artery bypass grafting; CCTA, coronary computed tomography angiography; CT, computed tomography; EAT, epicardial adipose tissue; ECG, electrocardiograph; LA-EAT, left atrial epicardial adipose tissue; NS, not specified; PAF, paroxysmal AF; POAF, post-operative AF; and TTE, transthoracic echocardiography.

3.7 FIGURE LEGEND

Figure 1. CONSORT Diagram of the Search Methodology

Figure 2. Comparison of Epicardial Fat in Prevalent AF Versus Sinus Rhythm

This analysis looks at the relation between epicardial fat volume and prevalent AF, comparing patients with pre-existing AF vs. those in sinus rhythm. Effects size presented as standardized mean difference (SMD) of EAT.

Figure 3. Association of Epicardial Fat with AF Presence

This comparison looks at the association of EAT with risk of AF presence. Effects were pooled as adjusted odds ratio (OR, *per* 1-SD increase in EAT) of AF (covariates: age, sex, obstructive sleep apnoea, type 2 diabetes mellitus, BMI, hypertension, heart failure, ischaemic heart disease, valvular heart disease, left atrial volume). The small squares represent effect sizes; the horizontal bars as 95% confidence intervals; and big diamond box as overall effect size estimate, respectively.

Figure 4. Comparison of Left Atrial Volume and AF

Figure 5. EAT Thickness and Prevalent AF

Figure 6. Total EAT Volume and Risk of Incident AF

Figure 7. Evaluation of Epicardial Fat and AF Progression

This analysis investigates epicardial fat in patients with non-paroxysmal AF versus paroxysmal AF. Effect size presented as SMD. **a**.)

Figure 8. Comparison of Left Atrial Epicardial Fat Volume in Non-paroxysmal Versus Paroxysmal AF

Figure 1. CONSORT Diagram of the Search Methodology

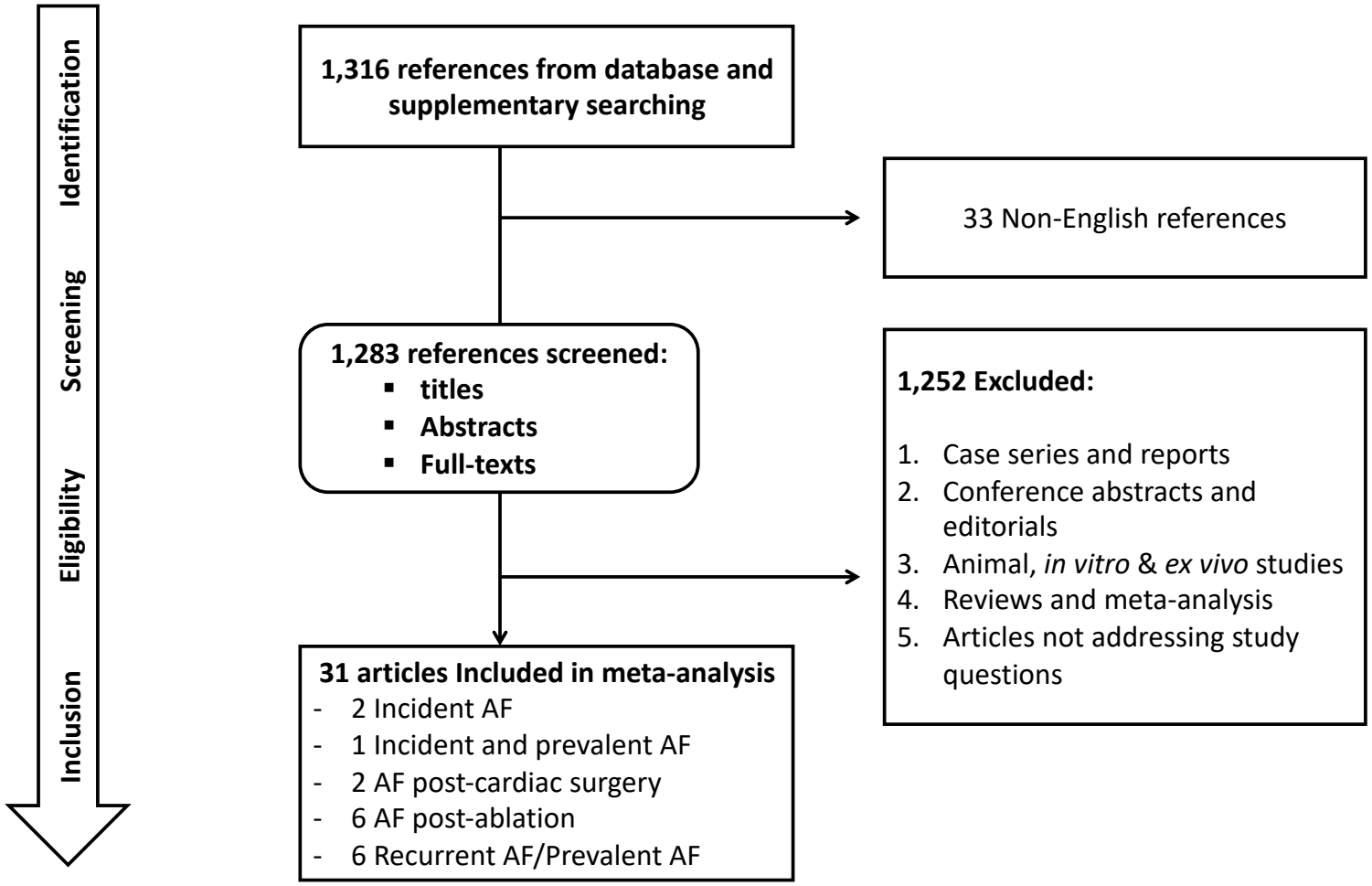


Figure 2. Comparison of Epicardial Fat in Prevalent AF Versus Sinus Rhythm

	AF			SR				Std. Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Random, 95% CI**
Al Chekakie et al. 2010	101.6	44.1	197	76.1	36.3	76	10.8%	0.60 [0.33, 0.87]
Greif et al. 2013	284.81	139.21	354	255.7	127.19	934	12.2%	0.22 [0.10, 0.35]
Kanazawa et al. 2014	148.8	46.1	120	113.9	32.5	120	10.8%	0.87 [0.61, 1.14]
Mahabadi et al. 2014	147.1	64.4	46	92.7	46.1	3809	10.5%	1.17 [0.88, 1.47]
Nagashima et al. 2011	185.6	76.1	40	138.3	45.2	37	8.4%	0.74 [0.28, 1.20]
Sevinc et al. 2017	54.33	23.4	58	42.99	20.8	74	9.8%	0.51 [0.16, 0.86]
Shin et al. 2011	83.8	26.8	80	67.2	23.1	80	10.2%	0.66 [0.34, 0.98]
Stojankovska et al. 2015	83.33	47	169	53	25	62	10.4%	0.72 [0.42, 1.01]
Tsao et al. 2011	29.9	12.1	68	20.2	6.5	34	8.8%	0.91 [0.48, 1.34]
Wong et al. 2011	299.5	160	102	162.7	46.4	20	8.0%	0.92 [0.43, 1.41]
Total (95% CI)			**1234**			**5246**	**100.0%**	**0.72 [0.49, 0.95]**

Heterogeneity: $Tau^2 = 0.11$; $Chi^2 = 57.75$, df = 9 ($P < 0.00001$); $I^2 = 84\%$

Test for overall effect: $Z = 6.07$ ($P < 0.00001$)

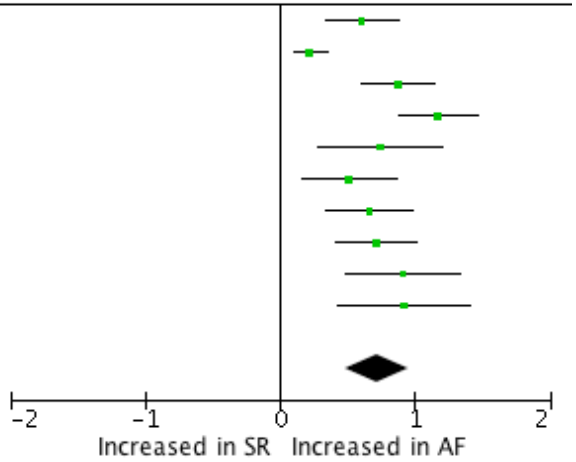


Figure 3. Association of Epicardial Fat with AF Presence

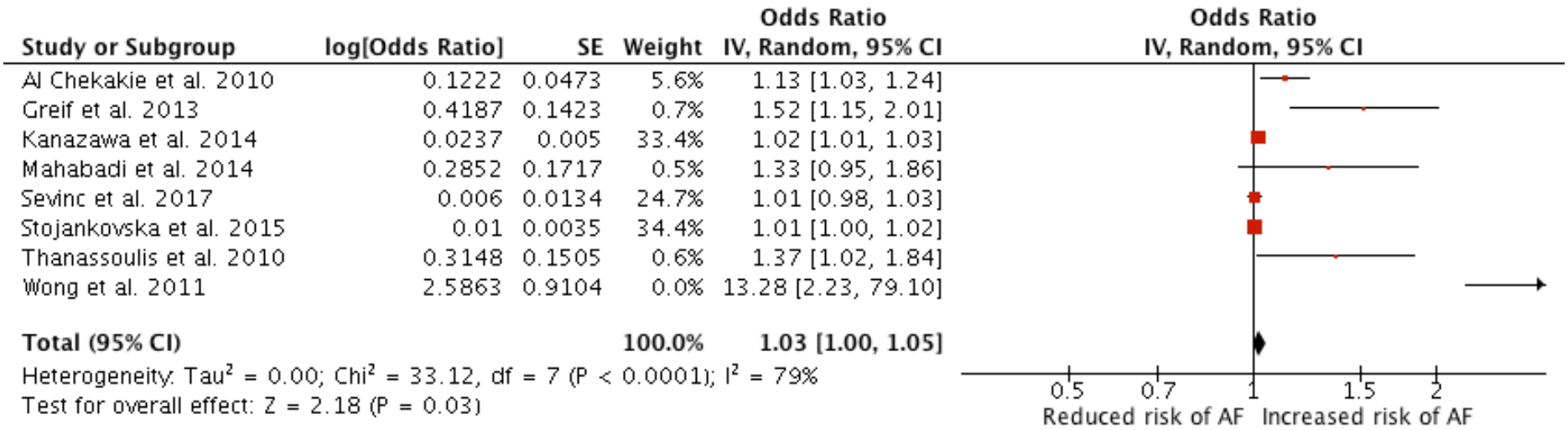


Figure 4. Comparison of Left Atrial Volume and AF

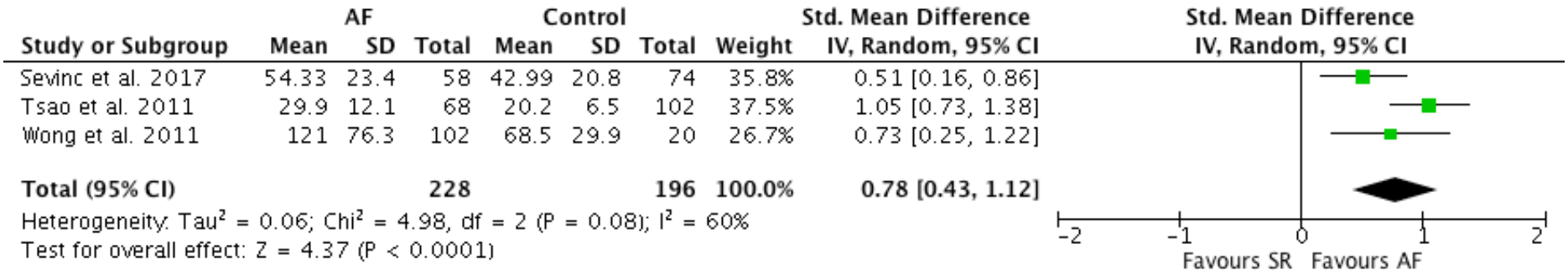


Figure 5. EAT Thickness and Prevalent AF

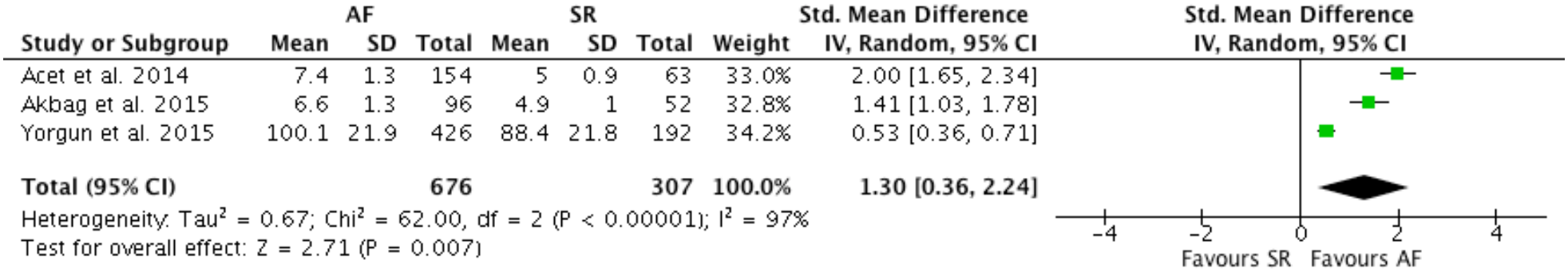


Figure 6. Total EAT Volume and Risk of Incident AF

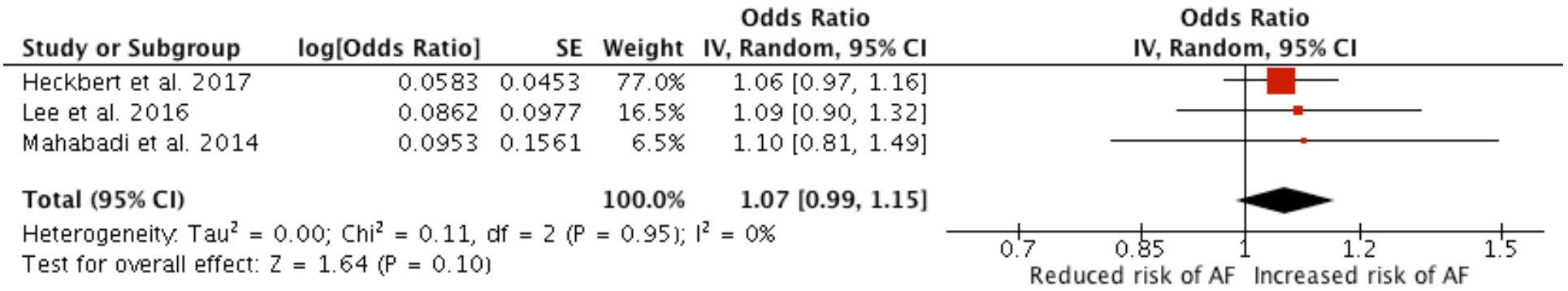


Figure 7. Evaluation of Epicardial Fat and AF Progression

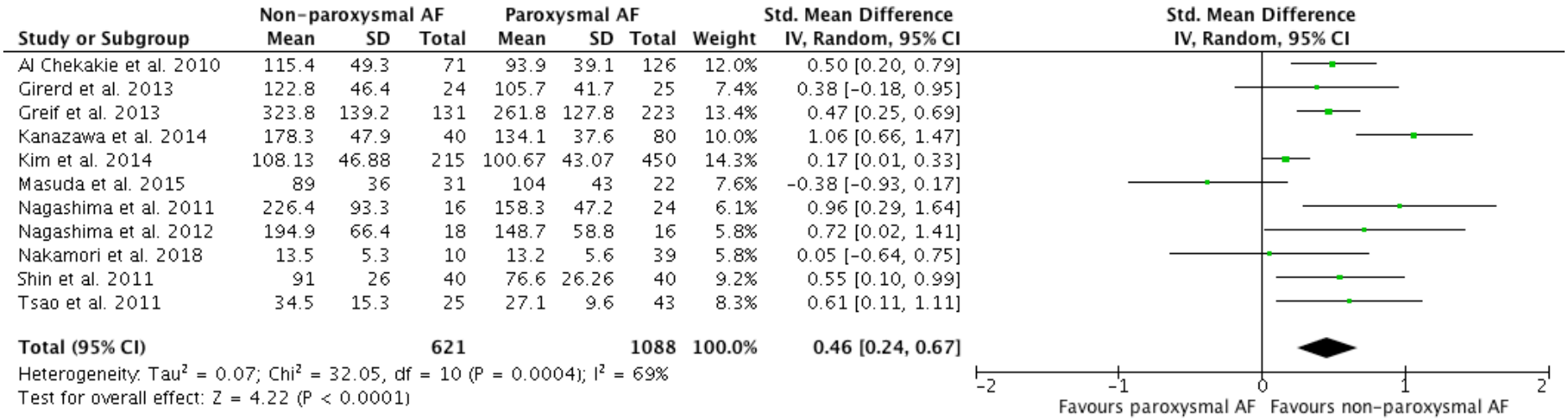


Figure 8. Comparison of Left Atrial Epicardial Fat Volume in Non-paroxysmal Versus Paroxysmal AF

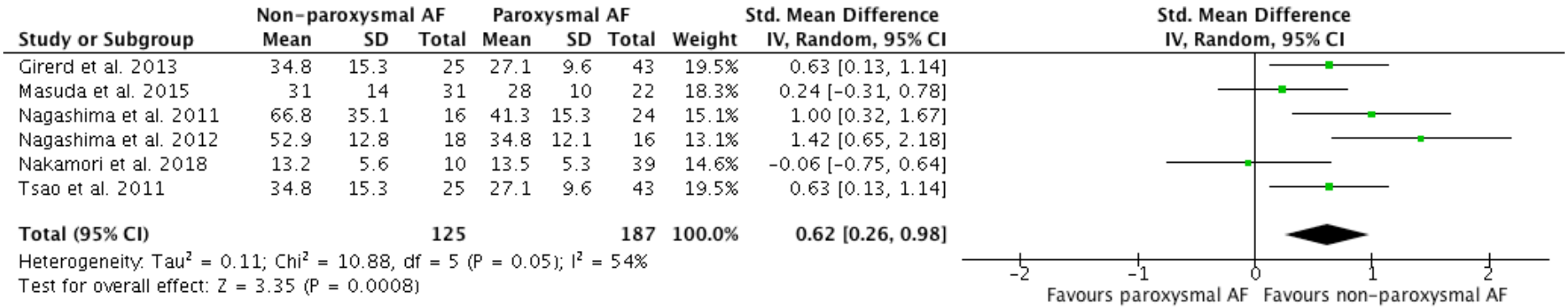


Figure 9. Comparison of Total Epicardial Fat Thickness in Non-paroxysmal AF Versus Paroxysmal AF

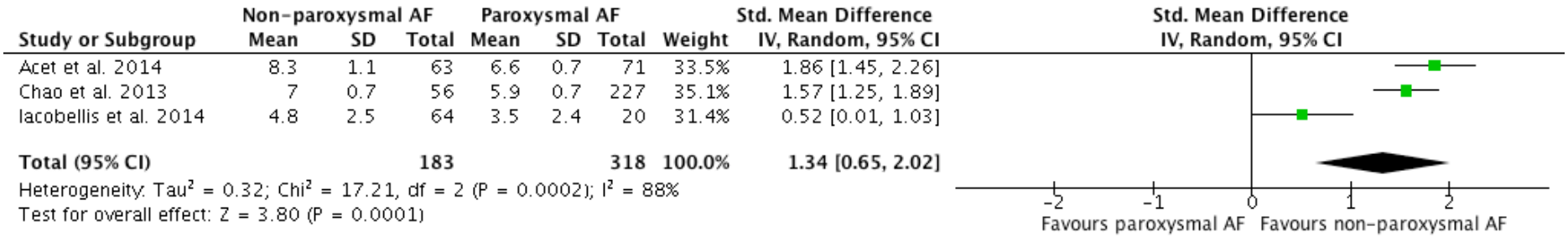


Figure 10: Evaluation of Epicardial Fat Volume in Recurrent AF

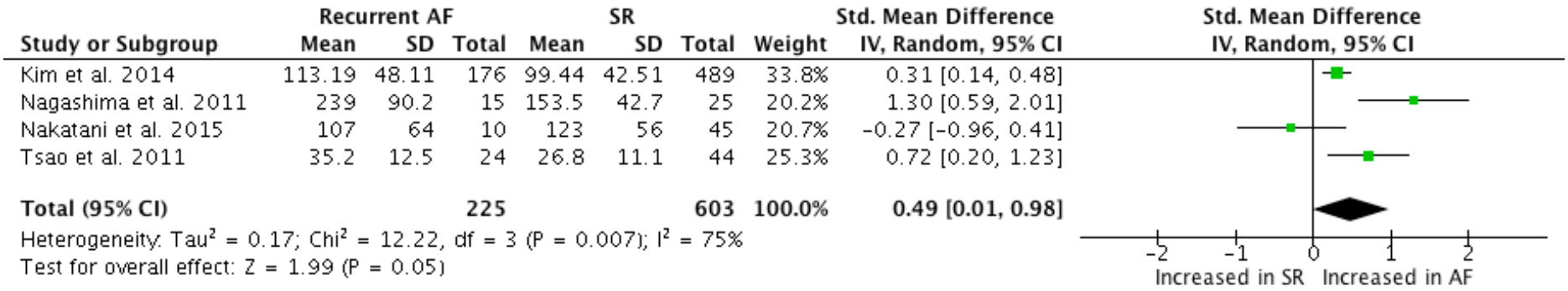


Figure 11. Epicardial Fat Volume and Risk of Recurrent AF

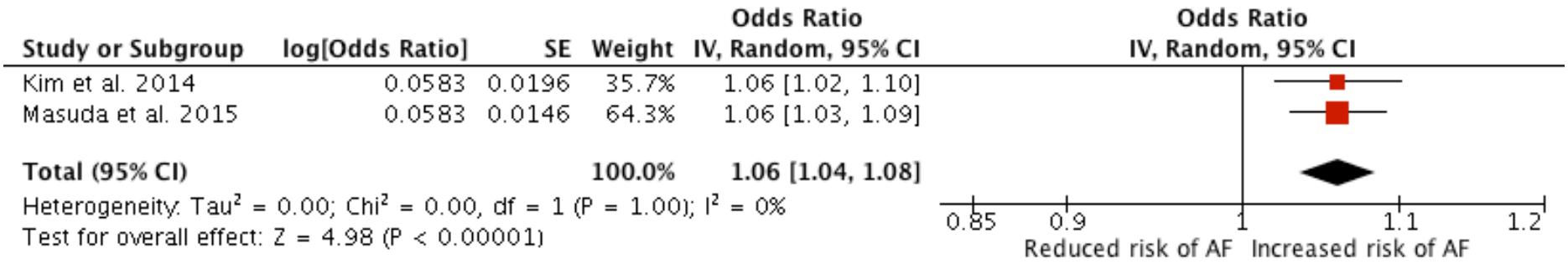


Figure 12. Epicardial Fat Thickness and AF Recurrence

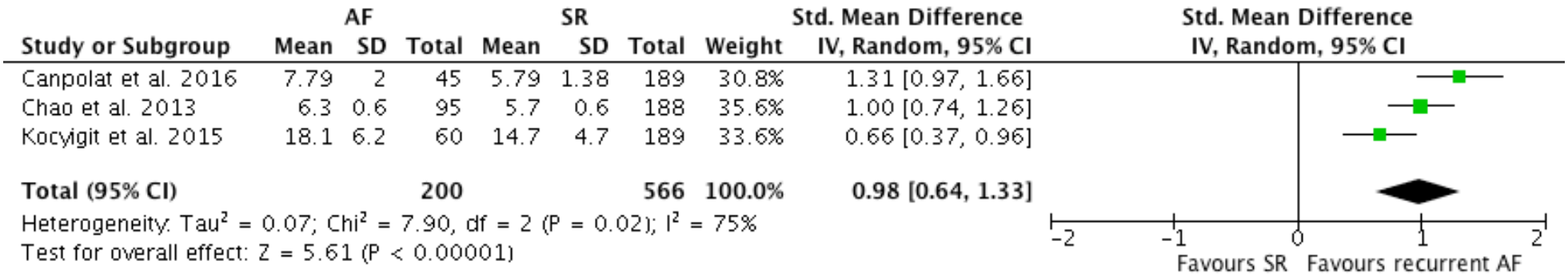


Figure 13. Comparison of Epicardial Fat and Post-operative AF

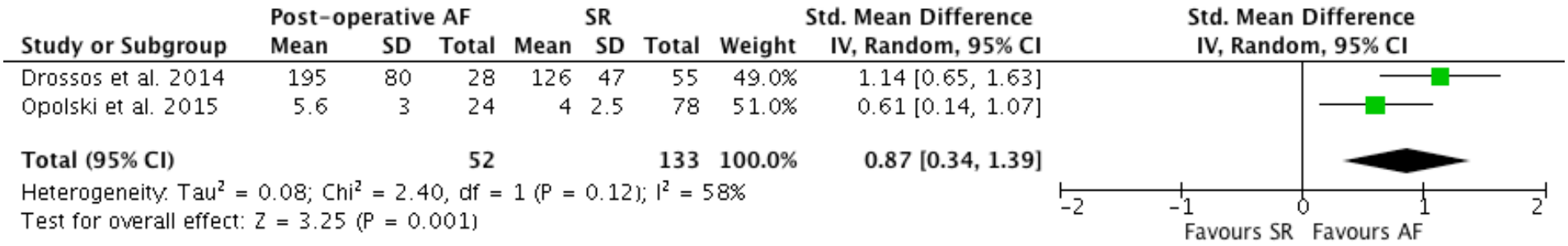
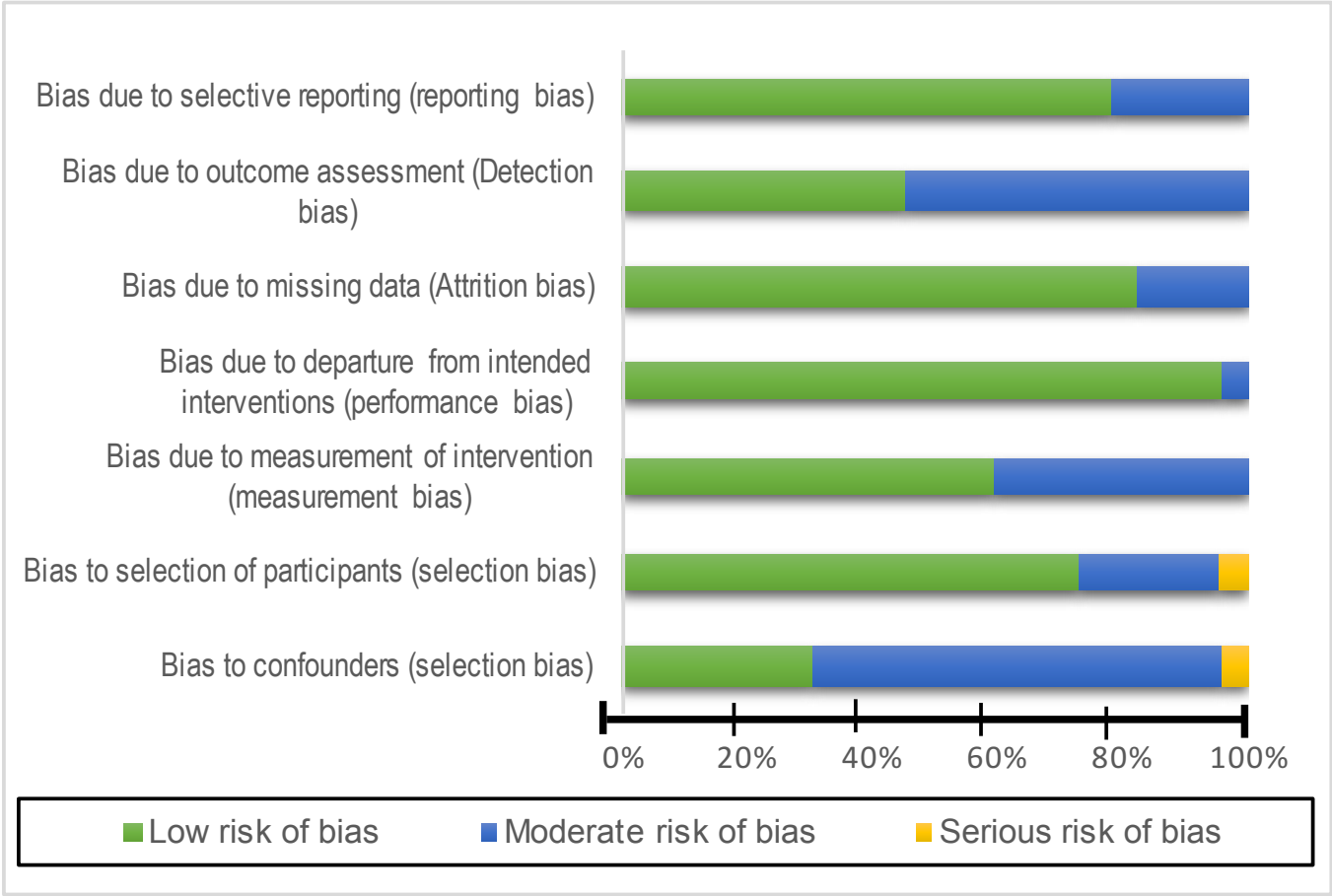


Figure 14. Risk of Bias Chart: A summary of Judgements Regarding 'Risk of Bias' Presented as Percentages Across all Included Studies



4. Chapter Four

Electrical and Electroanatomic Characterisation of the Atria in Obesity and Weight Fluctuation

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4.1 INTRODUCTION

Obesity has emerged as an important modifiable risk for atrial fibrillation (AF). Body mass index, a measure of overall adiposity, is demonstrated to predict excess risk of incident new-onset AF[261] and progression[416], recurrent post-ablation AF[417], and new-onset AF after cardiac surgery[418]. More recently, data has implicated epicardial adipose tissue (EAT) expansion, which occurs during weight gain, in the clinical link between obesity and AF.[263] However, the mechanisms associating EAT with AF risk have not been fully elucidated. While we have previously reported increased EAT in the obesity in both short term and long-term, we do not fully understand how ectopic fat relates with atrial electrical substrates

Furthermore, weight fluctuation, a common finding in the clinic, is has been implicated in adverse health conditions[343, 344]. For example, in the LEGACY study, long-term prospective study, we demonstrated benefit of weight loss in reducing the burden of recurrent AF in long-term follow up. However, up to 5% weight fluctuation significant reduction in the freedom from AF compared to linear weight loss.[345] The puzzling question remains as to what constitute the atrial substrate due to fluctuating weight. We hypothesis that weight fluctuation during weight loss will result in persistent atrial remodelling.

The aims of the current study were to: (1) characterise atrial electrical substrates in stable obesity; (2) characterise atrial electrical substrates due to weight fluctuation; (3) evaluate epicardial fat in weight fluctuations and compare these to obesity; and (4) evaluate the relation between epicardial fat remodelling and electrical substrates.

4.2 METHODS

4.2.1 Animals

Twenty-four Merino Cross Wethers sheep (*Ovis aries*) were studied in accordance with guidelines outlined in the "Australian Code for the Responsible Conduct of Research, 2007 (the 2007 Code)" adopted jointly by the National Health and Medical Research Council, the Australian Research Council and Universities Australia. The protocol and animals used herein were approved by both the animal research ethics committees of the University of Adelaide and the South Australian Health and Medical Research Institute, Adelaide, Australia, which adhere to the Guidelines for the Care and Use of Animals for Research Purposes.

4.2.2 Obese Ovine Model

Obesity was induced in 8 sheep using a previously well characterised protocol. In brief, sheep were commenced on a high-calorie diet for a period of 40 weeks and maintained in this state for another 40 weeks. Obesity induction was started at baseline, whereby healthy sheep with normal weight were put on a diet consisting of energy-dense soy-bean 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. Pellets were gradually introduced at 8% excess basal energy requirements and rationed to 70% of total dry-matter intake. Blood samples were periodically collected to ensure electrolyte and acid-base homeostasis.

4.2.3 Weight Fluctuation Model

Another group of 8 sheep was maintained as the weight fluctuation animal and were commenced on a four 20-week cycles of weight gain/weight loss. All animals were commenced on a high-calorie diet similar to obese sheep for a period of 20 weeks. Thereafter, sheep were maintained on high quality hay for another 20 weeks to induce weight loss, with energy-dense pellets rationed at just 0.75% of body weight. At the end of the 20 weeks, the cycle was repeated again. Blood samples were periodically collected to ensure electrolyte and acid-base homeostasis.

4.2.4 Lean Control Model

Eight age-matched sheep were maintained as controls at their baseline weight. To do this, high-quality hay was provided ad libitum, while energy-dense pellets were rationed at 0.75% of body weight. The nutritional content of food and housing conditions were identical for all three groups, with only the amount of food intake varying.

4.2.5 Animal Preparation

Animals were pre-acclimatized for at least 1 week before any surgery. Shorn weight was recorded immediately before surgery.

4.2.6 PROTOCOL

4.2.6.1.1 Haemodynamic Assessment

Invasive blood pressure (BP) monitoring was performed during the electrophysiology study. Left atrial (LA) and right atrial (RA) pressures were recorded.

4.2.6.1.2 Cardiac MRI

Before open chest surgery, animals underwent cardiac MRI using 1.5 Tesla (Siemens Sonata, MR Imaging Systems, Siemens Medical Solutions, Erlangen, Germany) with 10-mm slices through the ventricles without interslice gaps. To do this, animals were securely placed in the dorsal recumbent position for scanning. Mechanical ventilation was maintained, facilitating electrocardiogram-gated image acquisition with periodic breath holding. Analyses were performed offline by blinded operators by using the proprietary software QMass MR (Medis medical imaging systems, Leiden, The Netherlands). The following parameters were measured as previously described: Left ventricular chamber mass; LV ejection fraction (LVEF); Left atrial end-systolic volume (LA-ESv); LA end-diastolic volume (LA-EDv); right atrial end-systolic volume (RA-ESv); and epicardial fat volumes.

Quantification of EAT: Epicardial fat volumes were quantified using previously validated protocol.[413] Briefly, a 3D model was constructed from consecutive end-diastolic short-axis images using semi-automated software. Regions of adipose tissue were marked in each slice followed by linear interpolation of pixel intensities in spaces between consecutive image slices. Periatrial and periventricular fat were defined as any pericardial fat subtending the the right and left atria and ventricles and below the visceral pericardium, respectively. Total

volume of adipose tissue was calculated as a total volume of the 3D model and the mass estimated from volume measurements.

4.2.6.1.3 Electrophysiological Study

The electrophysiological study was carried out based on previously published methodology[182] and in the post-absorptive state under general anaesthesia. Briefly, venous access was obtained through the right femoral and left internal jugular veins. A 10-pole catheter with 2-5-2 mm inter-electrode spacing (Daig Electrophysiology, Minnetonka, MN) was advanced through the left internal jugular vein and positioned in the coronary sinus (CS). A conventional trans-septal puncture was performed using a BRK1 needle and SL0 sheath to access the left atrium.

Surface electrocardiogram (ECG) and bipolar endocardial electrograms were continuously monitored and stored on a computer based digital amplifier/recorder system for off-line analysis (LabSystem Pro, Bard Electrophysiology, Lowell, MA, USA). Intracardiac electrograms were filtered from 30 to 500 Hz, and measured with computer assisted calipers at a sweep speed of 200 mm/s. The following were conducted:

4.2.6.1.3.1 Effective Refractory Period Assessment

The effective refractory period (ERP) was performed using a rove catheter after the electroanatomical mapping study. All ERPs were evaluated at twice the diastolic threshold at cycle length (CL) of 400 ms using an 8-beat drive train followed by an extra-stimuli (S_2), which started with an S_2 coupling interval of 120 ms increasing in 5 ms increments. The ERP was defined as the longest coupling interval failing to propagate to the atrium. ERP was

measured from the following 8 sites: 1) RA appendage; 2) RA lateral wall, upper; 3) RA lateral wall, lower; 4) proximal CS; 5) distal CS; 6) LA appendage (LAA); 7) LA posterior wall; and 8) LA inferior wall.

4.2.6.1.4 Electroanatomical Mapping

Electroanatomic maps of the LA/RA were created in sinus rhythm using the CARTO (Biosense Webster) mapping system as previously published. This carried out using A 3.5-mm tip catheter (Navistar, Biosense Webster, Diamond Bar, California). The accuracy of the sensor position has been previously validated to 0.8 mm and 5^{o}. Briefly, the system records the surface ECG and bipolar electrograms filtered at 30 to 400 Hz from the mapping and reference catheters. Endocardial contact during point acquisition was facilitated by electrogram stability, fluoroscopy, and the catheter icon on the CARTO system. Points were acquired in the auto-freeze mode if they met the stability criteria in space (≤6 mm) and local activation time (LAT; ≤5 ms). Mapping was performed with an equal distribution of points using a fill-threshold of 15 mm. Collected points were edited offline. LAT was manually annotated to the peak of the largest amplitude deflection on bipolar electrograms. In the presence of double potentials, the LAT was annotated at the largest potential. If the bipolar electrogram displayed equivalent maximum positive and negative deflections, the maximum negative deflection on the simultaneously acquired unipolar electrogram was used to annotate the LAT.

Each point was binned according to location (region), fractionation (presence or absence), scar (presence or absence), and bipolar voltage amplitude to allow analysis in a mixed-effects model. Regional atrial bipolar voltage and conduction velocity were analysed

offline. The LA/RA maps were segmented for analysis, and the following parameters were assessed as previously described:

1. *Atrial conduction velocity:* To determine conduction velocity (CV), isochronal activation maps (5-ms interval) of the atria were created and regional CV measured in the direction of the wave-front propagation (i.e., least isochronal crowding). This was then assessed by averaging the distance between 3 to 5 pairs of points as a function of the difference in LAT. CV was measured in the following regions: 1) RA upper lateral wall; 2) RA lower lateral wall; 3) RA septal wall; 4) LA posterior wall; 5) LA inferior wall; and 6) LA lateral wall.
2. *Complex electrogram fractionation:* Electrograms with a duration ≥50 ms and 3 or more deflections crossing baseline were considered complex fractionated electrograms; and double potentials were potentials separated by an isoelectric interval and with a total electrogram duration ≥50 ms. For analysis, a fraction of the total number of fractionated/double points was utilized.
3. *Atrial voltage:* Low-voltage areas were defined as 3 contiguous points with a bipolar voltage <0.5 mV. Electrically silent areas (scar) were defined as 3 contiguous points with an absence of recordable activity or bipolar voltage amplitude <0.05 mV.

4.2.7 Statistical Analysis

Data were tested for normality using Shapiro-Wilk tests. Normally distributed continuous data were expressed as mean plus or minus standard deviation (SD) and analysed with ANOVA across groups (controls, obese and weight fluctuation). Skewed distributions were expressed as median and interquartile range (IQR) and medians tested using Mann-Whitney

U-tests, or Kruskal-Wallis tests. Nominal data was analysed by *Chi-square* tests of independence. Next, we fitted mixed-effect models to the data in order to compare conduction velocity and atrial refractory period across regions, chambers, and groups (control, obese and weight fluctuation), with two fixed effects at a time. To investigate LA regional patterns in both approaches, region (posterior LA, inferior LA and LA appendage) and group (control, obese and weight fluctuation) were modelled as fixed effects with an interaction term (region x group). RA regional patterns were similarly investigated. If a significant interaction was present, mixed-effects post-hoc test p-values were reported (with Sidak adjustment of alpha level). To determine the correlation of electrical remodelling with epicardial fat, we fitted bivariate linear regression and estimated Pearson coefficient (r) and r^2. Two-sided p-values ≤0.05 were considered statistically significant. All analyses were performed using SPSS version 25 (IBM SPSS Statistics, Chicago, Illinois) and GraphPad Prism version 7.0d (GraphPad Software, La Jolla, CA, USA).

4.3 RESULTS

4.3.1 Group Characteristics

The obese state was achieved over 80 weeks, with the obese group reaching peak weight (109.1±7.1 $kg.m^{-2}$) by the 40th week and sustained at the achieved weight for another 40 weeks. The weight fluctuation group reached obese state by 20 weeks; after this, they lost weight for 20 weeks, with obesity re-induced for another 20 weeks before finally undergoing another round of weight loss reaching a weight of 77.2±4.5 $kg.m^{-2}$. The control group maintained lean weight (76.1±4.5) over the 80-week period. By the end of 80 weeks, the obese sheep significantly increased their baseline weight to almost twice the control and

weight fluctuation levels ([p<0.001 for both] **Table 1**). The final weights of the controls and the weight fluctuation sheep were not significantly different from each other (p>0.05). The Electrolyte, acid-base, and glucose levels remained within their normal ranges throughout the over feeding process.

4.3.2 Electrophysiological Remodelling

Table 1 shows summary of electrical findings due to chronic weight gain and fluctuation.

4.3.2.1 Effective Refractory Period

Obesity resulted in reduction in mean atrial refractoriness compared to controls (p<0.001) and weight fluctuation (p=0.003), **Table 1**. There was no significant change in mean atrial ERP between fluctuating weight group and controls. Interestingly, ERP did not change significantly between the groups across all sites evaluated, except at right atrial appendage, where ERP in the obese animals was significantly abbreviated compared to controls ([130.1±37.6 ms vs. 169.3±53.1 ms], p=0.03) and weight fluctuation ([130.1±37.6 ms vs. 170.8±50.0 ms], p=0.02), see **Figure 1**.

4.3.2.2 Atrial Conduction

Figure 2 shows the endocardial CV across left and right atrial sites. In the left atrium, there was a significant reduction in the mean endocardial CV in the obese sheep compared to controls ([0.97±0.1 m/s vs. 1.26±0.1 m/s], p<0.001). This was found in LA posterior wall (LAPW: p=0.036), LA inferior wall (LAIF: p<0.001), and LA lateral wall (LALW: p<0.001). Similarly, obesity demonstrated significant reduction of endocardial CV in the right atrium.

The mean endocardial CV was reduced by 23% following sustained weight gain compared to maintaining baseline weight ([0.96±0.1 m/s vs. 1.24±0.1 m/s], $p<0.001$). Obesity also demonstrated regional conduction slowing in the RA, including RA upper lateral (RAUL: $p<0.001$), lower lateral (RALL: $p=0.008$), and septal walls (RASW: $p<0.001$), respectively.

When weight fluctuation was compared to lean controls, fluctuating weight was associated with significant persistent reduction in CV across both chambers. In the LA, mean CV was slower by 0.20 m/s ([1.06±0.1 m/s vs. 1.26±0.1 m/s], $p<0.001$) in the weight fluctuation cohort compared to controls. CV changes were seen across the posterior and inferior walls ([LAPW: $p=0.024$] and [LAIF: $p=0.002$]) but not in the lateral wall (LALW: $p=0.328$). In the RA, there was a 0.15 m/s reduction in mean CV in the weight fluctuation compared to controls ([1.09±0.1 m/s vs. 1.24±0.1 m/s], $p<0.001$). Regionally, slowed conduction was seen in the lower lateral and septal walls ([RALL: $p=0.0158$] and [RASW: $p=0.0145$]). In the upper lateral wall, conduction trended towards significant slowing with fluctuating weight ($p=0.063$).

Importantly, final weight loss in the weight fluctuation cohort resulted in mean CV being mildly increased in both the LA and RA. Regionally, while CV was comparable in the posterior and inferior LA, CV was greater in the lateral wall in the weight fluctuation compared to obese sheep. Regional CV was reduced in the upper lateral and septal walls of the RA in obese animals compared to weight fluctuation ([RAUL: $p<0.001$] and [RASW: $p<0.001$]). However, the lower lateral wall had similar CV in both groups ($p=0.963$).

4.3.2.3 Electrogram Fractionation

Obesity resulted in doubling of LA electrogram fractionation compared to controls ([24.7±3.6 mm vs. 12.1±2.9 mm], p<0.001), see **Table 1** and **Figure 3A**. With fluctuation in weight, LA fractionation was persistently increased compared to lean weight controls ([18.2±3.7 mm vs. 12.1±2.9 mm], p=0.006). Fractionated electrograms were found be 50.4% higher in the weight fluctuation group. On the other hand, when compared to stable obesity, weight fluctuation was associated with 26.3% lower fractionated electrograms in the LA ([24.7±3.6 mm vs. 18.2±3.7 mm], p=0.002).

4.3.2.4 LA voltage

See **Table 1** and **Figure 3B** for a summary of LA voltages in all three groups. The mean LA voltage in the obese sheep was 6.5±1.1 mV. In the controls, we found similar results (6.5±0.8), with no significant difference between the two groups (p=1.0). The mean LA voltage was slightly reduced in the weight fluctuation group compared to lean controls (5.8±1.1 mV vs. 6.5±0.8 mV). However, this was not significantly different between the two (p=0.387). Similarly, weight fluctuation did not result in significant reduction in LA voltage compared to stable obesity in the sheep models ([5.8±1.1 mV vs. 6.5±1.1], p=0.387).

4.3.3 Epicardial Adipose Tissue Remodelling

See **Table 1 and Figure 4** for summary of epicardial adipose tissue (EAT) quantified by cardiac MRI. In relation to the atria, epicardial adipose tissue was distributed adjacent to the LA posterior wall and atrioventricular groove (**Figure 4A**). Total cardiac EAT was greater in obese group compared to controls (p=0.037) and weight fluctuation (p=0.039). There was no

significant difference in total cardiac EAT depot between weight fluctuation and control groups (p=0.98). For the total atrial depot, there was slight increase in fat volume in obese group compared to controls but did not reach significance (p=0.2). The volume of total atrial EAT increased in obesity than did weight fluctuation group (p=0.02). No significant difference in LA EAT was noted for all groups. For RA EAT, depot volume did not significantly change between obese or weight fluctuation group and controls; however, obese animals showed almost 2-fold higher fat volume compared to weight fluctuation groups.

4.3.3.1 Relationship of Epicardial Fat with Electrical Remodelling

Table 2 and **Figures 5** to **7** summarise the linear regression results of epicardial fat and electrical substrates. We noted weak correlation between total cardiac EAT and LA voltage and electrogram fractionation, which did not reach statistical significance ($p>0.05$ for both). Again, there was no significant correlation between total EAT and CV and ERP ($p>0.05$ for both). Chamber-based sub-analysis did reveal any significant relation between EAT and any electrical parameters (see **Figure 5-7**).

4.4 DISCUSSION

4.4.1 Major Findings

The present study provides new mechanistic insights into the nature of fibro-fatty infiltrations as an evolving substrate for AF obesity and in fluctuating weight. Using a chronic ovine sheep model:

Compared to reference controls, atrial substrate due to chronic obesity was characterized by:

1. 23% significant reduction in atrial endocardial conduction velocities
2. More than 2-fold increased fractionated electrograms
3. 14% abbreviated mean atrial refractoriness
4. Nonsignificant change in left atrial voltage
5. 46% significantly expanded total cardiac epicardial fat volume

Compared to reference controls, atrial substrate due to weight fluctuation was characterised by:

1. 12% to 16% significant reduction in conduction velocities in the atria
2. 50.4% greater fractionated electrograms in left atrium
3. Non-significant changes in mean refractoriness and left atrial voltage
4. Nonsignificant change in total cardiac epicardial fat depots.

Compared to stable obesity, final weight loss in weight fluctuation was characterised by:

1. Significant weight reduction
2. Significant reductions of right atrial (48%), total atrial (35%), and total cardiac epicardial (29%) fat depots
3. 26.3% reduced fractionated electrograms
4. Mildly increased conduction velocity
5. 13% increased mean atrial ERP
6. Nonsignificant change in LA EAT, regional ERPs, and LA voltage

In summary, despite achieving weight loss, weight fluctuation during weight loss resulted in persistent atrial remodelling despite comparable volumes of epicardial fat depots as lean weight and showed similar atrial substrate to obesity, albeit to a lesser extent. These findings

are consistent with the clinical findings in the LEGACY study associated with weight fluctuation.[345]

4.4.2 Pro-arrhythmic Substrate Due to Obesity

Obesity is reported to associate with an increased risk of atrial arrhythmias in several epidemiological studies.[261, 416] Indeed, intensive research is underway to delineate the mechanisms that may mediate this sinister clinical link. Given the low-grade inflammation, neurohumoral activations, and autonomic imbalance seen during chronic obesity, it is likely that they might drive the formation of AF substrate in obesity.[181, 340] Consistent with this, in an earlier model of short-term obesity, we showed significant atrial enlargement, induction of fibrosis, and inflammatory infiltrates.[181] Obesity was associated with greater expressions of pro-fibrotic markers, including endothelin (ET)-1, ET receptors (ET_AR & ET_BR), transforming growth factor-beta 1 and platelet-derived growth factor in dose-dependent fashions. Mahajan et al[182] corroborated these findings by demonstrating LA enlargement, increased interstitial fibrosis with accompanying pro-fibrotic TGF-β1 expression using a sustained model of obesity, induced by high-calorie feeding for 72 weeks.

Impairment in electrophysiological properties of the atrium is an important further requisite for AF substrate formation.[11] We previously reported global biatrial endocardial remodelling characterized by conduction abnormalities, fractionated electrograms, and increased propensity for AF during sustained weight gain, highlighting that obesity could induce electrical remodelling.[182] This is well in line with current findings. Nonetheless, unlike previous studies, we failed to see association of obesity with reduced posterior LA endocardial voltage, which we speculate could be blunted by chronic age of our models.

4.4.3 Epicardial Fat and Pro-arrhythmic Substrate

Epicardial fat expansion is an important factor involved in the pro-arrhythmic substrate formation. Experimental studies have shown cardiac MRI assessed EAT expansion as a common consequence of obesity, which underscores its role in explaining the clinical link between obesity and AF.[181, 182] In the present study, we found significantly higher total cardiac fat depot but comparable atrial depots with controls. This is in line the findings of Wong et al[263], reporting non-significant association between peri-atrial EAT and AF burden and risk of recurrence after catheter ablation. The author did note, however, that the AF burden or recurrence is driven by periventricular depots.[263] Furthermore, we showed that weight fluctuation is associated with reduction of EAT depots as compared to obesity.

4.4.4 Weight Fluctuation and AF Substrate

In multiple lines of clinical reports, weight loss has been associated with changes in hormonal balance in patients.[343, 344] This is postulated to impact both physiological and pathophysiological changes.[419] These compensatory mechanisms were investigated by Sumithran et al[344] in a population of postmenopausal women (BMI: 27-40 $kg.m^{-2}$). After weight loss induced by calorie restriction, there was significant increase in appetite regulating factors, such ghrelin, gastric inhibitory polypeptide, and pancreatic polypeptide. One year after weight reduction, the changes did not return normal value at baseline, thus it highlights that the compensatory changes in these factors could cause weight relapse. Hypothetically, abnormal release of adipokines like leptin could both directly and indirectly pose great arrhythmogenic risk. Importantly, our current data sheds light on the potential nature of the

atrial substrate due to periodic fluxes in weight, a common clinical finding in patients.[344] Despite having comparable weights with reference controls, weight fluctuation sheep demonstrated LA enlargement, and abnormal atrial conduction and fractionated electrograms, without change in atrial endocardial voltage or refractoriness. More importantly, we noted that the electro-structural remodelling during weight fluctuation is less severe as compared to stable obesity. We believe these observations may explain the findings in the LEGACY study.

4.4.5 Study Limitations

Several limitations are worth noting in the current study. First, there was no segmentation performed for atrial EAT due to the limited resolution of CMR. We believe that this could have hidden important details on the regional distribution of EAT, which may have prevented conclusion on regions that may of important clinical relevance. Segmentation of EAT, as reported by Nagashima et al[302], would have allowed us to determine EAT location that most likely mediate the observed elecro-structural substrate seen therein. It should be noted that those investigators used computed tomography as their imaging modality, which demonstrates much higher resolution.

4.4.6 Potential Clinical Implications

These findings provide a mechanistic basis for the clinical associations reported between expansion of epicardial fat and AF. EAT may represent a useful risk marker to identify patients at an increased AF risk which could allow a more personalized risk stratification. EAT could be a promising target for atrial fibrillation, a concept recently tested in patients

who underwent pulmonary vein isolation wherein treatment with atorvastatin led to reduction in EAT volume. Furthermore, these results provide mechanistic basis for the perils of periodic weight fluctuation often encountered in clinical settings. Although weight loss is likely to reduce EAT, fluxes in weight could remodel patients' atria by induction of fibro-fatty infiltrations and pathologic modulation of contractile units in myocytes. Further studies are warranted to explore potential therapies targeting intramyocardial fat cell depositions and to determine whether their modulation could constitute a treatment target for primary and secondary prevention of AF.

4.5 CONCLUSIONS

The findings herein demonstrate that chronic obesity induces significant reduction of endocardial CV remodelling, doubling of electrogram fractionation, abbreviation of refractoriness, and expansion of epicardial fat, without significant change in LA voltage. Despite comparable weight with controls, weight fluctuation was associated with significant conduction slowing and LA electrogram fractionation, without any significant change in mean refractoriness, LA voltage, or total cardiac fat depots. Compared to stable obesity, sheep with weight fluctuation showed significant weight loss, lower RA and atrial EAT, mildly greater endocardial CV, and increased mean atrial ERP, without significant change in LA EAT and LA voltage. This data demonstrates that weight fluctuation results in residual endocardial biatrial and electrophysiological remodelling.

4.6 TABLES

Table 1. Anthropometric, and Structural and Electrophysiological Characteristics of Obese, Weight Fluctuation and Control Sheep

Parameter	Control	Obese	Weight fluctuation	p-value		
				Obese vs. controls	Weight fluctuation vs. controls	Obese vs. weight fluctuation
Weight (kg)	76.1±4.5	109.1±7.1	77.2±4.5	<0.001	0.960	<0.001
LA EAT (mL)	4.3±1.3	5.0±2.0	4.2±1.1	0.666	0.987	0.556
RA EAT (mL)	5.1±1.1	6.9±2.9	3.6±0.9	0.174	0.262	0.006
Total atrial EAT (mL)	9.5±1.3	12.0±4.5	7.8±1.3	0.228	0.490	0.023
Total cardiac EAT (mL)	163.5±22.1	238.4±77.1	169.4±47.7	0.037	0.980	0.039
LA voltage (mV)	6.5±0.8	6.5±1.1	5.8±1.1	1.000	0.387	0.387
CV, LA (m/s)	1.26±0.11	0.97±0.08	1.06±0.13	<0.001	<0.001	0.026
CV, RA (m/s)	1.24±0.10	0.96±0.09	1.09±0.11	<0.001	<0.001	<0.001
ERP mean (ms)	178.6±38.1	153.4±32.7	173.1±31.5	<0.001	0.640	0.003
ERP, LA (ms)	162.0±33.2	140.0±21.8	161.0±23.5	0.140	0.929	0.074
ERP, RA (ms)	179.0±22.8	157.0±31.7	180.0±15.9	0.165	0.911	0.081
ERP, CS (ms)	199.0±40.8	171.0±23.5	182.0±41.1	0.115	0.438	0.490
LA fractionation (mm)	12.1±2.9	24.7±3.6	18.2±3.7	<0.001	0.006	0.002
LV ejection fraction (%)	41.6 (8.7)	44.9 (8.3)	45.1 (10.2)	0.710	0.336	0.694

Table 2. Relationship Between Total Cardiac Epicardial Fat and Structural and Electrical Substrates

Parameter	Pearson *r*	r^2	*p*-value
LA voltage (mV)	0.262	0.069	0.251
CV, LA (m/s)	-0.141	0.020	0.542
CV, RA (m/s))	-0.236	0.056	0.331
ERP mean, LA (ms)	-0.217	0.047	0.345
ERP mean, RA (ms)	-0.216	0.047	0.347
LA fractionation (mm)	0.312	0.097	0.158

4.7 FIGURE LEGENDS

Figure 1. Distribution of Effective Refractory Periods Across Atrial Sites and Animal Groups

ERP was not changed as results of obesity or weight fluctuation across atrial chambes and regions, except in right atrial appendage. **LAPW**, left atrial posterior wall; **LAIF**, left atrial inferior wall; **LAA**, left atrial appendage; **RAUL**, right atrial upper lateral wall; **RALL**, right atrial lower lateral wall; **RAA**, right atrial appendage; **Prox CS**; proximal coronary sinus; **Dist CS**; distal coronary sinus; CN, controls; OB, obese; WF, weight fluctuation.

Figure 2. Changes in Conduction and Conduction Heterogeneity Caused by Weight Fluctuation

Panel A: Endocardial conduction velocity measured six atrial sites. Panel B: Epicardial conduction velocity measured across two corners in control, obese and weight fluctuation sheep. Panel C: Mean conduction heterogeneity indices of control, obese and weight fluctuation sheep measured across two epicardial plaque corners. **LAPW**, left atrial posterior wall; **LAIF**, left atrial inferior wall; **LALW**, left atrial lateral wall; **RAUL**, right atrial upper lateral wall; **RALL**, right atrial lower lateral wall; **RASW**, right atrial septal wall; **C1**; corner 1; **C2**; corner 2; **CN**, controls; OB, obese; WF, weight fluctuation; CHI, conduction heterogeneity index; ns, $p>0.05$.

Figure 3. Left Atrial Voltage and Electrogram Fractionation Changes Induced by Weight Fluctuation

Panel A: Left atrial electrogram fractionation of control, obese and weight fluctuation animals. **Panel B:** Left atrial voltage. #, $p<0.05$: obese vs. controls; &, $p<0.05$: obese vs. weight fluctuation; *, $p<0.05$: weight fluctuation vs. controls; **CN**, controls; OB, obese; WF, weight fluctuation.

Figure 4. Epicardial Fat Volume Quantified by Cardiac MRI

Panel A: Distribution of fat on freshly harvested sheep heart. Fat is mostly abundant around the atrioventricular groove and interventricular sulcus. Adipose could also be observed around the atrial appendages (white arrowhead showing the left appendage). **Panel B:** Representative CMR images in long-axis view, demonstrating the distribution of epicardial adipose tissue in the three study groups; atrial EAT depots are highlighted with contours. **Panel C:** Box and Whisker charts

demonstrating quantified volumes of EAT from the four cardiac chambers; showing total cardiac, total peri-atrial, left and right atrial EAT volumes, respectively. **CN**, controls; OB, obese; WF, weight fluctuation.

Figure 5. Relationship Between Epicardial Fat and Effective Refractory Period

Panel A: Correlation between total peri-atrial epicardial fat and mean left atrial ERP. **Panel B:** Correlations between right atrial EAT and right atrial ERP.

Figure 6. Relationship Between Epicardial Fat and Atrial Conduction

Panel A: Correlation between total epicardial fat and conduction velocity in the left atrium.

Panel B: Correlations between epicardial fat and conduction velocity in the right atrium.

Figure 7. Relationship Between Epicardial Fat and Fractionated Electrogram and Atrial Voltage

Panel A: Correlation between total epicardial fat and electrogram fractionation in left atrium.

Panel B: Correlations between epicardial fat and voltage in the left atrium.

Figure 1. Distribution of Effective Refractory Periods Across Atrial Sites and Animal Groups

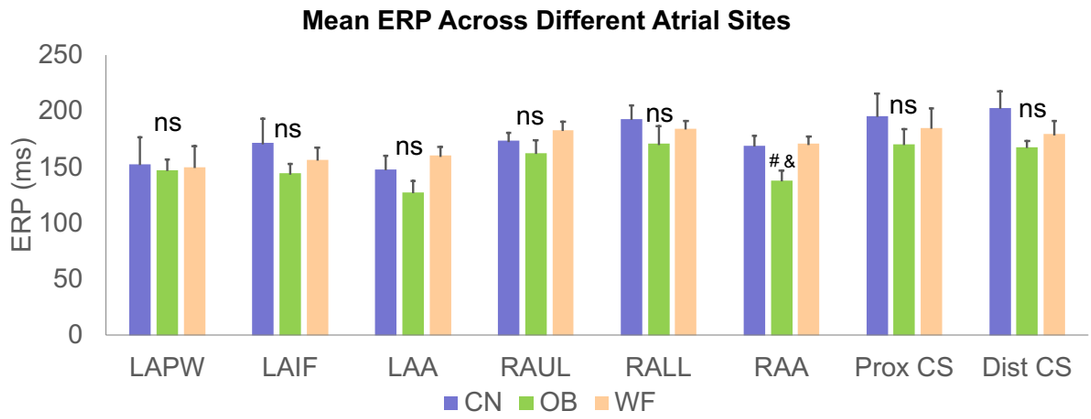


Figure 2. Changes in Conduction and Conduction Heterogeneity Caused by Weight Fluctuation

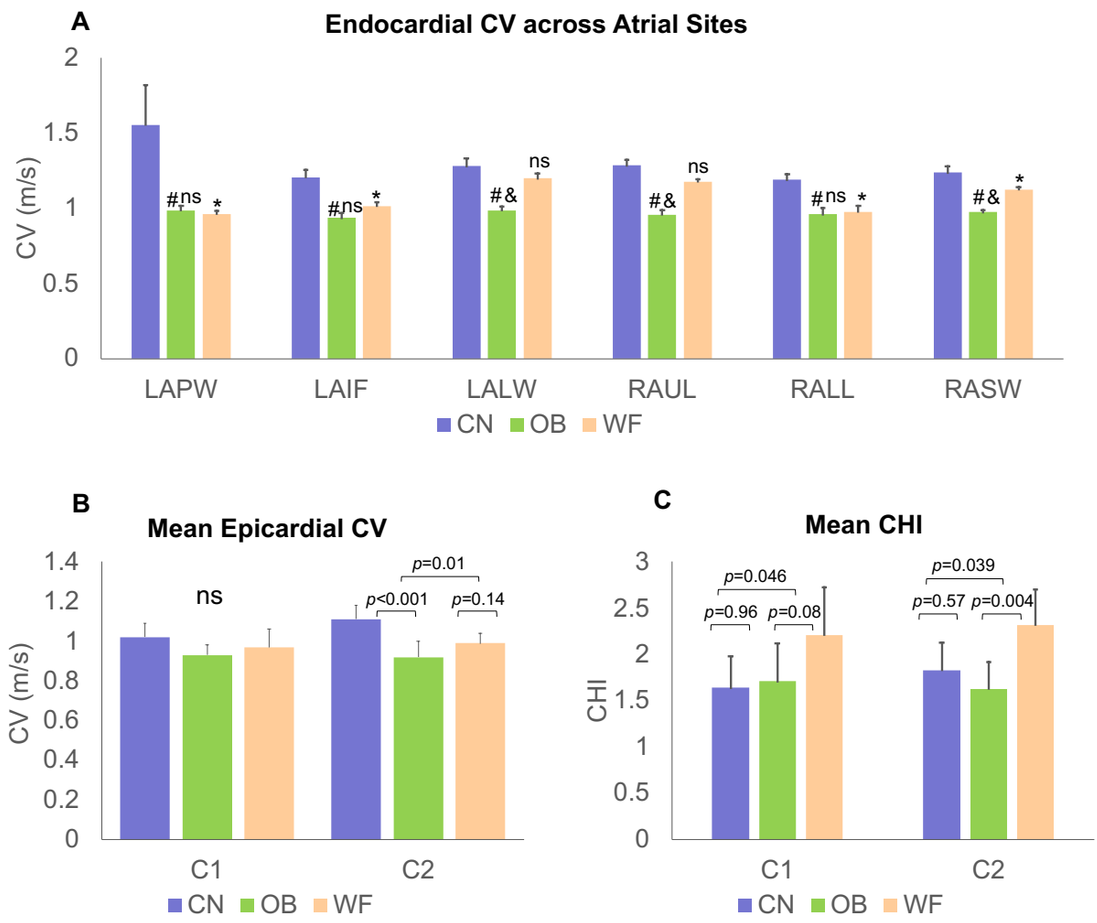


Figure 3. Left Atrial Voltage and Electrogram Fractionation Changes Induced by Weight Fluctuation

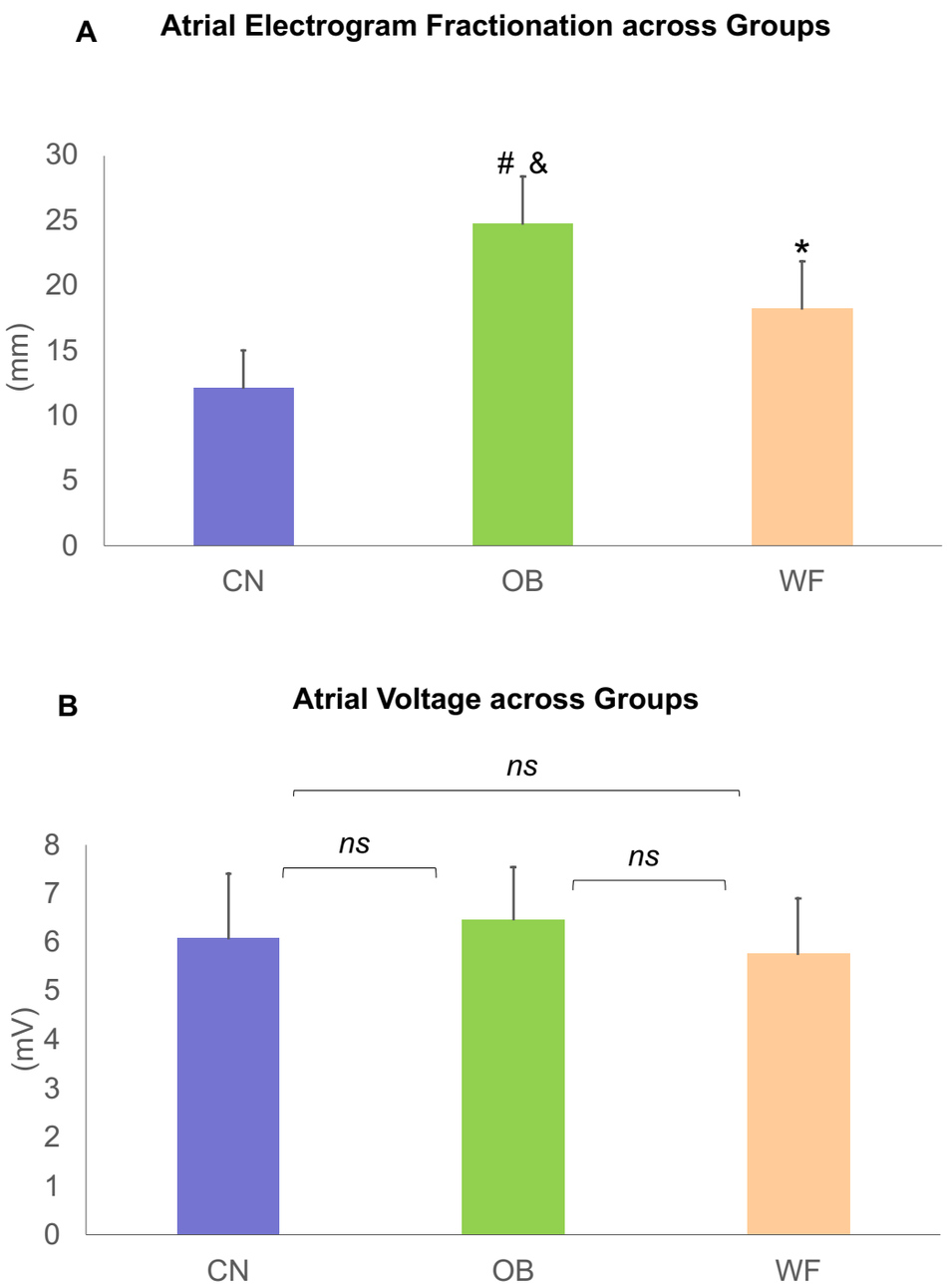


Figure 4. Epicardial Fat Volume Quantified by Cardiac MRI

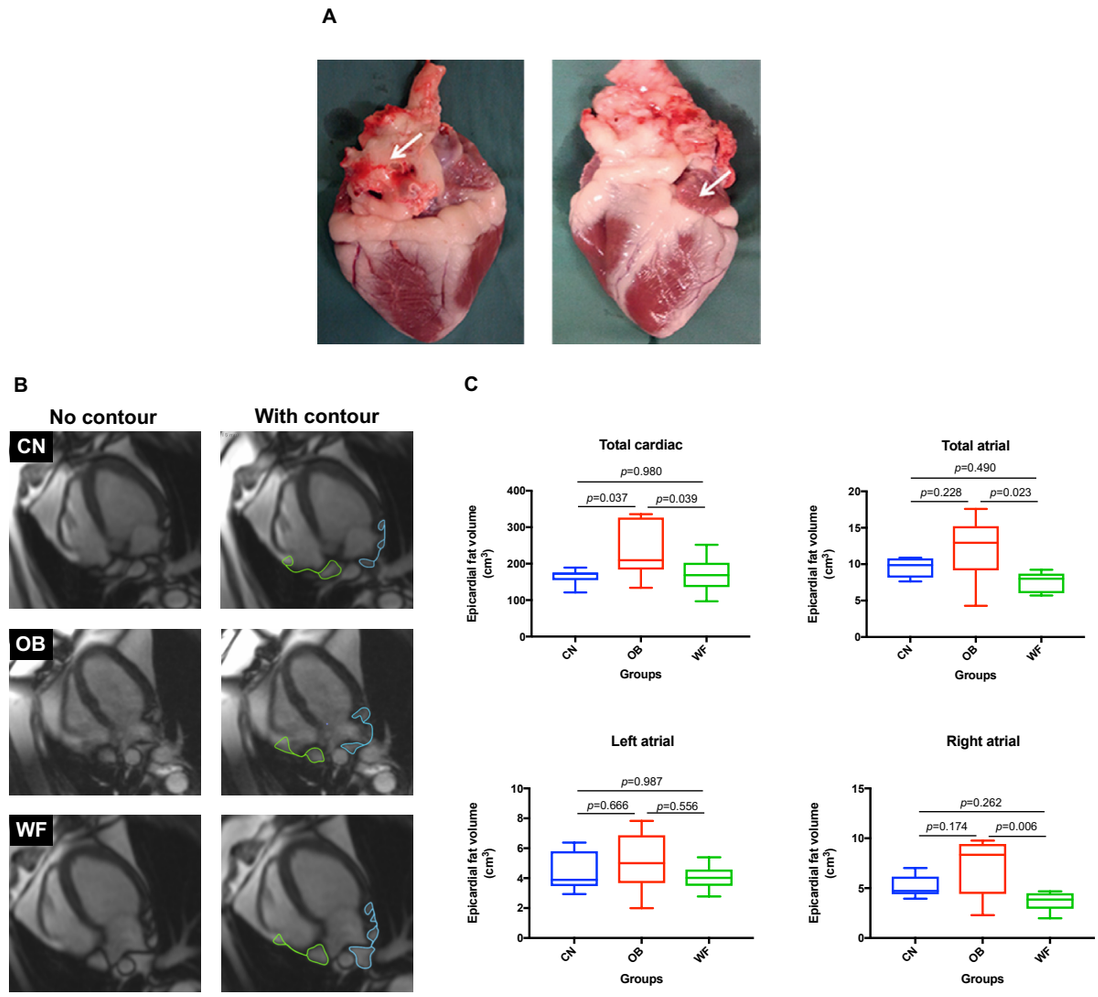


Figure 5. Relationship Between Epicardial Fat and Effective Refractory Period

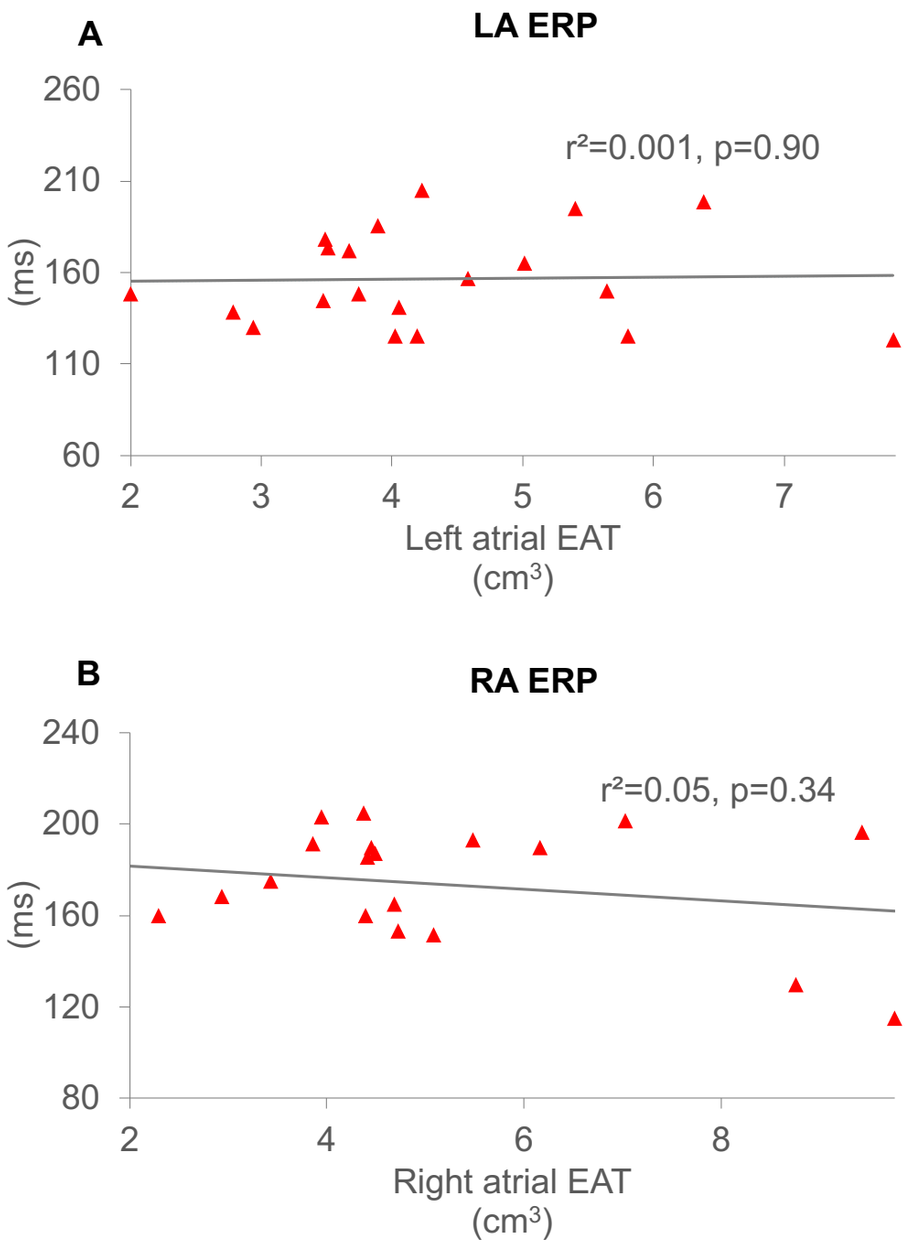


Figure 6. Relationship Between Epicardial Fat and Atrial Conduction

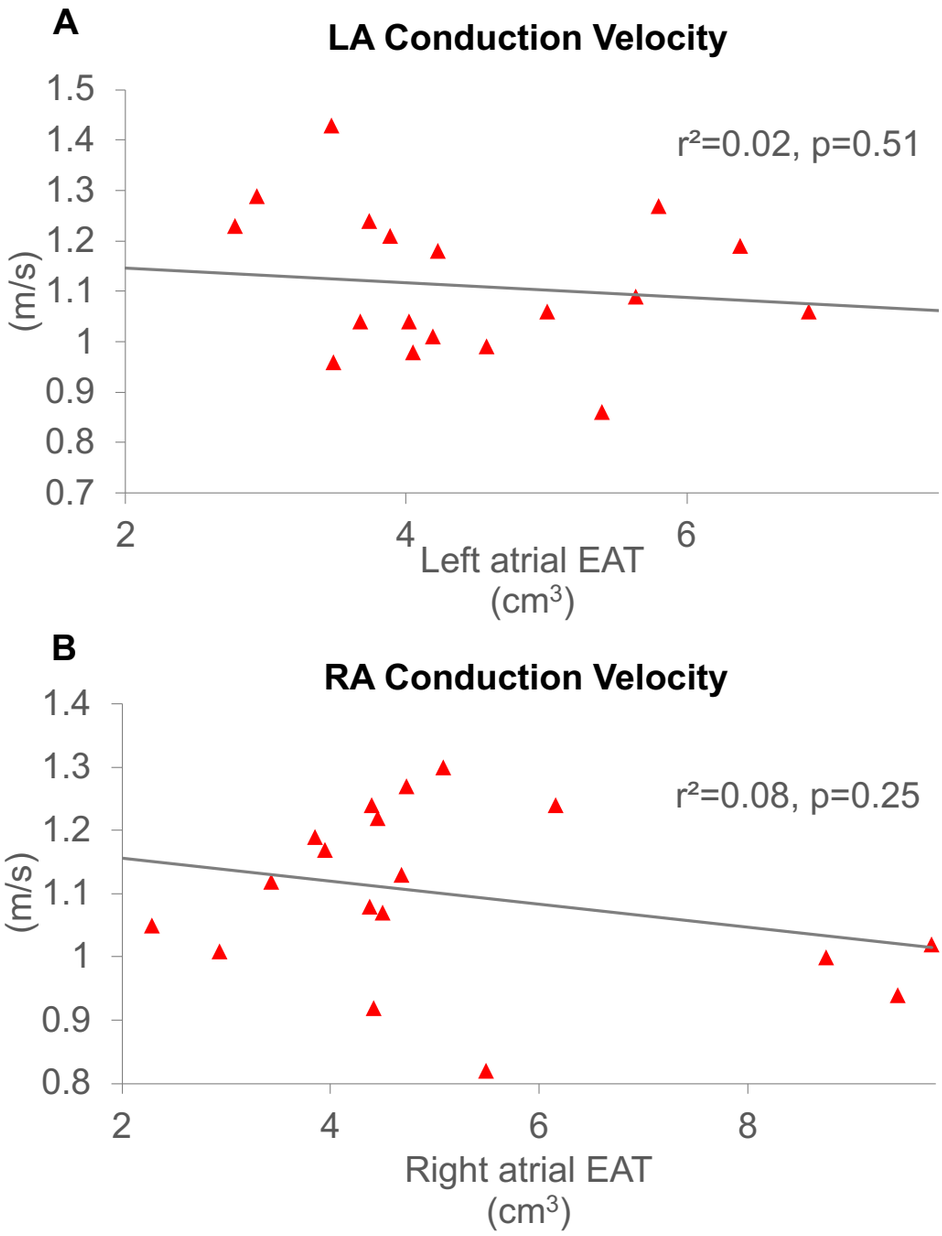
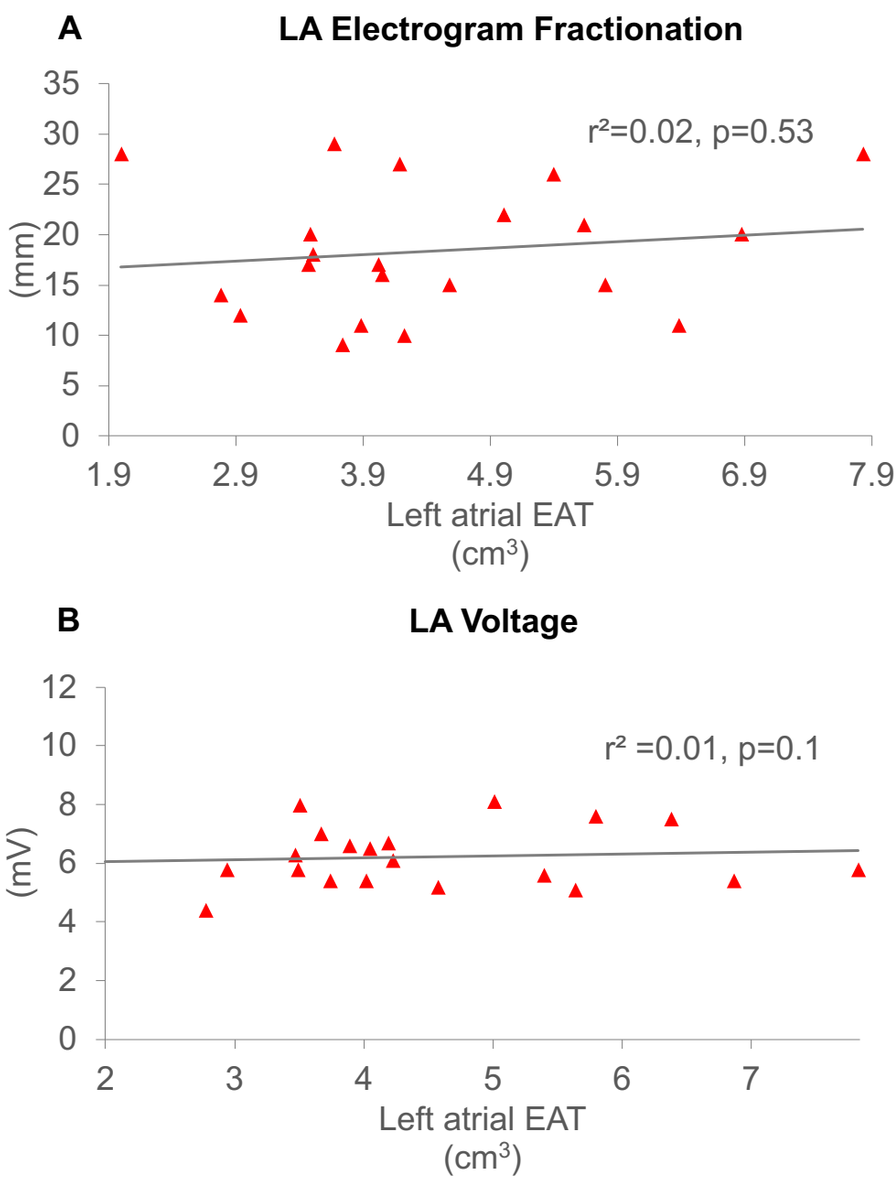


Figure 7. Relationship Between Epicardial Fat and Fractionated Electrogram and Atrial Voltage



5. Chapter Five

Cellular Mechanisms of Epicardial Fat in Obesity and Weight Fluctuation – Fibrofatty Infiltrations, Myofibrillar Remodelling and Lipid Imaging

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5.1 INTRODUCTION

Epicardial adipose tissue (EAT) has emerged as an important factor for formation of the atrial pro-arrhythmic substrate. EAT expansion, which occurs during weight gain, predicts increased risk of atrial fibrillation (AF)[182], poorer outcomes following catheter ablation[263] and bypass grafting[409], and correlates with greater risk of thromboembolic events[304, 305, 420]. Moreover, this association is reported to be independent of traditional measures of obesity, indicating that EAT can influence AF risk independently of obesity.

Recent advances have implicated epicardial fat in ectopic focal mechanisms and re-entrant substrates requisite for the initiation and perpetuation of AF.[11, 35, 119, 122, 128, 137] EAT has been associated with structural abnormalities promoting AF, such as induction of atrial fibrosis[278], atrial enlargement/stretch[181, 182], and inflammation[271, 272, 388]. We have also demonstrated relations between the ectopic fat and electrical substrate in a clinical model of obesity. Nonetheless, there is great paucity of data characterising the cellular and ultrastructural changes that may mediate EAT-induced atrial remodelling.

Consequently, therapeutic recourses targeting adiposity and limiting expansion of EAT are exciting research endeavours. In fact, weight loss has been established to reduce the burden of AF, with dose-dependent effects reported.[334, 345] However, recent clinical evidence has challenged this notion given that obese individuals often experience oscillation in weight.[344] For example, weight fluctuation was shown to offset the beneficial effects of weight loss, with more than 5% weight fluctuation in associated with twofold greater risk of arrhythmia recurrence after ablation.[345] The puzzling question remains as to what constitute the atrial substrate due to these fluxes in weight. We hypothesised that the periodic weight gains during weight fluctuation will result in fibrofatty infiltrations similar to obesity but that the degree of EAT remodelling may not be as severe.

In the present study, we aimed to characterise ultrastructural changes responsible for EAT-induced atrial remodelling; spatial distribution of lipids due to weight fluctuation and obesity; and evaluate the relation between these changes with atrial electrical and structural substrates.

5.2 METHODS

5.2.1 Animals

Twenty-four Merino Cross Wethers sheep (*Ovis aries*) were studied in accordance with guidelines outlined in the "Australian Code for the Responsible Conduct of Research, 2007 (the 2007 Code)" adopted jointly by the National Health and Medical Research Council, the Australian Research Council and Universities Australia. The protocol and animals used herein were approved by both the animal research ethics committees of the University of Adelaide and the South Australian Health and Medical Research Institute, Adelaide, Australia, which adhere to the Guidelines for the Care and Use of Animals for Research Purposes.

5.2.2 Obese Ovine Model

Obesity was induced in 8 sheep using a previously well characterised protocol. In brief, sheep were commenced on a high-calorie diet for a period of 40 weeks and maintained in this state for another 40 weeks. Obesity induction was started at baseline, whereby healthy sheep with normal weight were put on a diet consisting of energy-dense soy-bean 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. Pellets were gradually introduced at 8% excess basal energy requirements and rationed to 70% of total dry-matter intake. Blood samples were periodically collected to ensure electrolyte and acid-base homeostasis.

5.2.3 Weight Fluctuation Model

Another group of 8 sheep was maintained as the weight fluctuation animal and were commenced on a four 20-week cycles of weight gain/weight loss. All animals were commenced on a high-calorie diet similar to obese sheep for a period of 20 weeks. Thereafter, sheep were maintained on high quality hay for another 20 weeks to induce weight loss, with energy-dense pellets rationed at just 0.75% of body weight. At the end of the 20 weeks, the cycle was repeated again. Blood samples were periodically collected to ensure electrolyte and acid-base homeostasis.

5.2.4 Lean Control Model

Eight age-matched sheep were maintained as controls at their baseline weight. To do this, high-quality hay was provided ad libitum, while energy-dense pellets were rationed at 0.75% of body weight. The nutritional content of food and housing conditions were identical for all three groups, with only the amount of food intake varying.

5.2.5 Animal Preparation

Animals were pre-acclimatized for at least 1 week before any surgery. Shorn weight was recorded immediately before surgery.

5.2.6 PROTOCOL

5.2.6.1 Body Composition

Dual-energy x-ray absorptiometry scans were performed to accurately determine total body fat in the animals.

5.2.6.2 Haemodynamic Assessment

Invasive blood pressure (BP) monitoring was performed during the electrophysiology study. Left atrial (LA) and right atrial (RA) pressures were recorded.

5.2.6.3 Cardiac MRI

Before open chest surgery, animals underwent cardiac MRI using 1.5 Tesla (Siemens Sonata, MR Imaging Systems, Siemens Medical Solutions, Erlangen, Germany) with 10-mm slices through the ventricles without interslice gaps. To do this, animals were securely placed in the dorsal recumbent position for scanning. Mechanical ventilation was maintained, facilitating electrocardiogram-gated image acquisition with periodic breath holding. Analyses were performed offline by blinded operators by using the proprietary software QMass MR (Medis medical imaging systems, Leiden, The Netherlands). The following parameters were measured as previously described: Left ventricular chamber mass; LV ejection fraction (LVEF); Left atrial

end-systolic volume (LA-ESv); LA end-diastolic volume (LA-EDv); and right atrial end-systolic volume (RA-ESv).

5.2.6.4 STRUCTURAL CHARACTERISATION

Following the electrophysiological study, the sheep were maintained under general anaesthetic. Thereafter, they were euthanised by lethal dose of phentobarbitone injection, with samples taken for detailed histological and ultrastructural analyses.

5.2.6.4.1 Histological Assessment

Following euthanasia, atrial tissues were isolated from the LAA and RAA, perfusion-fixed with 4% paraformaldehyde and immersed in 10% buffered formalin. Tissue sections were processed and embedded in paraffin wax. Fixed blocks were cut into 5-µm serial sections and stained with haematoxylin and eosin (H&E) and Periodic Acid Schiff (PAS), respectively.

5.2.6.4.1.1 Fibrofatty Infiltration Assessment

The fibrofatty infiltration of the atria by was confirmed in Oil O Red preparations and H&E stained sections, and assessed on Masson's Trichrome stained sections as previously published. Slides were scanned by NanoZoomer digital image scanner and viewed on NDP.view 2 (Hamamatsu Photonics K.K., Japan). 6 sections were photographed at 100 µm (20x) magnification and processed in ImageJ (National Institutes of Health, USA). The following parameters were assessed:

1. *Grade infiltration:* Fatty infiltrates were graded by the extensive nature of the infiltration using scoring algorithm modified from previously published protocol (**Figure 1**). The algorithm ran on 1- to 4-point grade scale. Grading was done for the most severe infiltrate per section and repeated for obese, weight fluctuation and control sheep. The grades of 6 sections were taken and averaged (presented as mean per animal and number of sections per grade).

2. *Percent infiltration:* The percent area covered by all fibrofatty infiltrates per sections was measured and expressed as percentage of the total section area. This was done by manual segmentation, which was defined in the region of interest (ROI) manager of ImageJ.
3. *Total area of infiltration:* The total area of fibrofatty infiltrate was assessed by manual segmentation (ROI manager). This was done for the same subset of infiltrate used for determining grade infiltration.
4. *Number of adipocyte infiltrates:* The largest infiltrate (same used for grade infiltration and area of fibrofatty infiltration) was used to determine the number of adipocytes per infiltrate per section. This was determined in 6 to 10 sections and averaged.
5. *Adipocyte size:* Individual adipocyte characteristics were assessed by manual segmentation in the ROI manager (ImageJ). The following parameters were determined: the average area of adipocytes ($pixel^2$); the average thickness of adipocytes per infiltrate measured by Ferret's diameter (pixel); and perimeter of each adipocyte (pixel). The Ferret's diameter was determined as the longitudinal distance across each adipocyte – longest distance between two points across the adipocyte.
6. *Collagen area in infiltrate:* The amount of collagen in infiltrate was determined to show the extent fibrotic remodelling of the infiltrate. The collagen fibres were traced by manual segmentation in ROI manager (ImageJ) and measured in pixel.

5.2.6.4.1.2 Myolysis Assessment and Glycogen Accumulation

To quantify the degree of myolysis (sarcomere loss), 5-µm sections were stained with periodic acid Schiff (PAS). This was with the understanding that the loss of sarcomeric myofibrils results in gradual accumulation of the cytoplasmic space by glycogen molecules, which stain with a magenta colour on PAS against a toluidine blue background. Fifteen image sections per site were photographed at 25 µm distance (80x magnification) for a total of 8 animals per group. Assessment of myolysis was only done by counting number of myocytes with intact nucleus in the plane of the section. The grading of myolysis was performed as previously published[122] but with modification, as follows:

- Myocytes with <10% glycogen accumulation of the cytosol were considered to have no myolysis

- Myocytes with 10% to 25% glycogen accumulation were considered to be mildly myolytic
- Myocytes with >25% to 50% glycogen accumulation were considered to be moderately myolytic
- Myocytes with >50% glycogen accumulation were considered to be severely myolytic

The percentage of total myolysis was expressed as: (Number of myocytes with >10% glycogen accumulation/Total myocyte count) x 100%.

5.2.6.5 Matrix-Assisted Laser Desorption Ionization Imaging Mass Spectrometry

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry imaging (MALDI MSI) was used to determine spatial distribution of lipids in the atrial tissues in obesity and weight fluctuation. Following non-survival experiments, hearts were harvested and dissected and snap-frozen in liquid nitrogen and stored in -80˚C until further use.10-µm thick sections were cut at -20. To assist with desorption and ionisation, tissue sections were coated with 3 mg of *α-cyano*-4-hydroxycinnamic acid (αCHCA) matrix by sublimation. A laser was used to ionise the lipids and electric field was applied until they reached the detector. The laser set to an intensity of 150 a.u. and fired for 1.5 seconds per location at a repetition rate of 200 Hz. A spatial resolution of 60 µm was selected and a mass range of *m/z* 50-990 was acquired. MALDI Images were obtained using a MALDI Synapt HDMS Mass Spectrometer (Waters Corporation) in MS mode. Images were visualised using Biomap (Novartis).

5.2.7 Statistical Analysis

Data were tested for normality using Shapiro-Wilk tests. Normally distributed continuous data were expressed as mean plus or minus standard deviation (SD) and analysed with ANOVA across groups (controls, obese and weight fluctuation). Skewed distributions were expressed as median and interquartile range (IQR) and medians tested using Mann-Whitney U-tests, or Kruskal-Wallis tests. Nominal data was analysed by *Chi-square* tests of independence. In the case of skewed distribution (i.e., total myolysis assessment with PAS staining), data were log-

transformed before further analysis. To determine the correlation of myolysis with structural substrates, we fitted bivariate linear regression and estimated Pearson correlation coefficient (r) and r^2. Two-sided p-values ≤0.05 were considered statistically significant. All analyses were performed using SPSS version 25 (IBM SPSS Statistics, Chicago, Illinois) and GraphPad Prism version 7.0d (GraphPad Software, La Jolla, CA, USA).

5.3 RESULTS

5.3.1 Group Characteristics

After 80 weeks of high-calorie feeding, the obese group demonstrated significant weight gain compared to controls (109.1±7.1 $kg.m^{-2}$ vs. 76.1±4.5 $kg.m^{-2}$), see **Table 1**. The weight fluctuation group reached obese state by 20 weeks; after this, they lost weight for 20 weeks, with obesity re-induced for another 20 weeks before finally undergoing another round of weight loss reaching a weight of 77.2±4.5 $kg.m^{-2}$. The final weights of the controls and the weight fluctuation sheep were not significantly different from each other (p>0.05). The Electrolyte, acid-base, and glucose levels remained within their normal ranges throughout the over feeding process.

5.3.2 Structural, Functional and Haemodynamic Remodelling Blood Pressure

Changes in systolic BP are presented in **Table 1**, respectively. Obesity resulted in significant increase BP compared to controls (BP [93.2±14.3 vs. 79.4±5.0], p=0.034). Systolic BP changes were comparable in the weight fluctuation and lean controls (BP [80.8±10.1 vs. 79.4±5.0], p=ns). We observed higher systolic BP in the obese groups as compared to weight fluctuation (93.2±14.3 vs. 80.8±10.1), which trended towards significance (p=0.053).

5.3.2.1 Atrial Volume

Tables 1 & 2 provide summary of chamber characteristics of the study groups. The volume of the left atrium (LA) was increased (p=0.001) by 32% (53.6±5.8 cm^3 vs.40.6±3.0 cm^3) during

sustained obesity. Similarly, weight fluctuation resulted in significant increase in left atrial volume compared to controls (LA Volume [48.5±6.6 cm^3 vs. 40.6±3.0 cm^3], p=0.025). We found the change LA volume to weight fluctuation to be less severe than stable obesity (LA volume [53.6±5.8 cm^3 vs. 48.5±6.6 cm^3], p<0.001). In the RA, we observed significant difference in chamber volumes across the three groups (p>0.05). Furthermore, there was no significant change between obese and controls in volumes at the end of diastole or systole in all comparisons (p>0.05 for all). The same was true for weight fluctuation (p>0.05). We also did not find any significant change in LV ejection fractions across all groups(p>0.05).

5.3.2.2 Atrial Pressure

Table 1 and **Figure 2** provide summary of haemodynamic characteristics of the study groups. Both obesity and weight fluctuation caused elevation in LA pressure. We observed more than 2-fold increased mean LA pressure compared to controls (Pressure [8.5±1.9 mm Hg vs. 3.7±0.9 mm Hg], p<0.001). With weight fluctuation, the sheep demonstrated 1.5 times higher mean LA pressure than lean controls (Pressure [5.7±1.8 mm Hg vs. 3.7±0.9 mm Hg], p=0.02), though this was less severe than in stable obesity (Pressure [8.5±1.9 mm Hg vs. 5.7±1.8 mm Hg], p=0.02). We further explored diastolic and systolic pressure changes, see **Figure 2A**. Obesity resulted in increased LA systolic and diastolic pressures compared to control (systolic [11.1±2.0 mm Hg vs. 6.7±0.9 mm Hg], p=0.002; diastolic [6.0±1.8 mm Hg vs. 2.1±1.1 mm Hg], p<0.001) or weight fluctuation groups (systolic [11.1±2.0 mm Hg vs. 7.7±3.0 mm Hg], p=0.015; diastolic [6.0±1.8 mm Hg vs. 3.6±1.0 mm Hg], p=0.007). On the other hand, LA systolic and diastolic pressures did not significantly change with weight fluctuation compared to lean controls (systolic [7.7±3.0 mm Hg vs. 6.7±0.9 mm Hg], p=0.002; diastolic [3.6±1.0 mm Hg vs. 2.1±1.1 mm Hg], p<0.001).

In the right atrium, obesity resulted in more than 2-fold greater mean right atrial (RA) pressure and lean controls (p<0.01), **Table 1**. Similarly, higher mean RA pressure was noted in the obese group compared to weight fluctuation group (p=0.048). We found RA systolic and diastolic pressure to be higher in obese sheep compared to controls and animals with weight fluctuation, see **Figure 2B**. Compared with controls, weight fluctuation did not result in greater

mean RA pressure ($p<0.05$ for all). Additionally, the systolic and diastolic pressures were significantly changed weight fluctuation in comparison to lean controls.

5.3.3 Fibrofatty Infiltration

Results for the fibrofatty infiltrations are summarised in **Table 2** and **Figures 3 & 4.** Due to chronic age of our model, the controls did show some appreciable infiltration of atrial myocardium by fat cells (**Figure 3A**, left inset). However, with obesity and weight fluctuation, the animals demonstrated extensive and widespread fatty infiltrations (**Figure 3A,** mid & right). The most severe infiltrates were characterised by excessive presence of adipocytes and inflammatory cells (**Figure 3B**, arrowheads). More intriguingly, there were presence of giant syncytia-looking fat cells likely indicative of cell fusions, which were more common in weight fluctuation group (**Figure 3C**, arrowheads). Both the obese group and weight fluctuation had greater average grade infiltrations compared to controls ($p<0.05$, **Figure 4A**). There was progressive increase in the number of obese and weight fluctuation animals as the infiltrate becomes more severe ($p = 0.001$, **Figure 4B**). There was no significant difference between obese and weight fluctuation groups ($p >0.05$ **Figure 4A**). The findings were consistent across both atrial chambers. When the region of infiltration was studied more comprehensively, only weight fluctuation was associated with significant greater adipocyte number than controls (**Figure 4D**). In the LA, collagen content of the infiltrate was not different between obese and controls ($p = 0.16$) but was significantly greater in weight fluctuation group compared to controls ($p = 0.042$). In the RA, there was greater collagen deposition in the infiltrates in the obese group than controls ($p = 0.05$, **Figure 4C**).

5.3.3.1 Fibrofatty Infiltrations and Electrical Remodelling

See **Table 3** and **Figure 5-7** for the summary of the linear regression of fibrofatty infiltrations and electrical substrate. Mean atrial fibro-fatty infiltration was correlated with LA and RA CV's and LA electrogram fractionations ([LA CV: $r^2 = 0.35$, $p = 0.006$], [RA CV: $r^2 = 0.52$, $p = 0.001$] and [fractionated electrogram: $r^2 = 0.50$, $p <0.001$] **Table 3**), but not LA voltage or ERP ($p > 0.05$ for both). We further evaluated chamber-based correlations. Consistent with results for

mean atrial infiltration, LA mean infiltration was correlated with LA fractionated electrograms and LA CV but not LA voltage ($p > 0.05$, **Figure 7C**) or LA mean ERP ($p > 0.05$, **Figure 5D**). The correlation was better with fractionated electrograms than with LA CV ([LA fractionation: $r^2 = 0.51$, $p < 0.001$], [$r^2 = 0.38$, $p = 0.004$] **Figures 6A & 7A**). RA mean infiltration was also correlated with RA CV ($r^2 = 0.45$, $p = 0.003$, **Figure 7**), but like atrial mean atrial infiltration, it did not correlate with RA mean ERP ($p = 0.37$, **Figure 7**).

5.3.3.2 Myolysis

As can be seen in **Figure 8 panel A**, most myocytes in the control group maintained their sarcomeric architecture as indicated by low cytosolic glycogen; only few had mild to moderate myolysis. With sustained obesity, the animals showed signs of extensive deterioration of myofibres and replacement of the contractile units by glycogen. Weight fluctuation exhibited similar impairment of contractile units, albeit to a lesser extent. In the LA, the percent total myolysis (mild + moderate + severe) increased in the obese group to more than 2.5-fold those in the control group (95.8±2.0% vs. 38.3±16.3%, $p < 0.001$) and by 30% of the weight fluctuation group (95.8±2.0 vs. 70.2±9.3%, $p = 0.001$), **Table 2** and **Figure 8 panel B**. The total myolysis in weight fluctuation groups increased to almost twice those in the controls (70.2±9.3% vs. 38.3±16.3%, $p = 0.012$), **Table 2** and **Figure 8 panel B**. In the RA, both obese and weight fluctuation animals showed greater total myolysis than controls ($p = 0.001$ for both), but comparable myolysis between both groups ($p = 0.92$), see **Table 2** and **Figure 8 panel B**.

Furthermore, there were observations of progressive severity of myolysis in the obese and weight fluctuation animals, see **Figure 9**. Myocytes with severe myolysis (>50% of cytosol covered by glycogen) were very few in the reference control group but greatly increased in both obese and weight fluctuation groups. Myocytes with less severe degree of myolysis became more prevalent in the controls and less so in the stable obesity and weight fluctuation groups.

5.3.3.3 Association of Myolysis with Structural and Functional Substrates

See **Table 4** and **Figure 10-11** for the summary of the linear regression of fibrofatty infiltrations and structural and functional substrates. Mean atrial myolysis was positively correlated with: body weight (r^2=0.39, p=0.025); atrial volume ([LA: r^2=0.37, p=0.009], [not with RA: r^2 = 0.09, p=0.23]; atrial pressures ([LA: r^2=0.40, p<0.01], [RA: r^2=0.28, p=0.03]); but not with systolic BP nor LV ejection fraction (p>0.05). We further evaluated chamber-based correlations. Consistent with results for mean atrial myolysis, both LA and RA myolysis did not correlate with systolic BP (p= 0.1 and 0.09). LA myolysis showed strong positive correlation with LA volume (r^2=0.51, p<0.01), meanwhile a negative correlation was found with RA myolysis and RA volume, though this was not significant (p=0.09). Additionally, both LA and RA myolysis were correlated with left and right atrial pressures ([LA: r^2=0.47, p=0.007] and [RA: r^2=0.26, p=0.04]), but not with LVEF (p>0.05), **Figures 12 & 13**.

5.3.3.4 Lipid Remodelling by Matrix-Assisted Laser Desorption Ionization Imaging Mass Spectrometry

Please refer to **Figure 14** for the lipid maps. We identified five lipid groups that had the most abundant spectral peaks: 184.06, 205.99, 760.57, 264.25, and 454.36, respectively (**Figure 14 panel B**). The *m/z* of 184.06, which corresponds to phosphatidylcholine (PC)/sphingomyelin headgroup, was abundant in the myocardium but not in epicardial layer, with most intensity seen in controls. Similar findings were observed for *m/z* of 760.57 (PC 34:1). *m/z* of 205.99, which could not be identified in the present pilot study, was more associated with fat regions. Differential expression and abundance were also noted for the *m/z* of 454.36, which again could not be identified, and was only observed in the obese myocardium. No further quantitative analysis was done due to the pilot nature of the present study, which was aimed to investigate the potential usefulness of MALDI imaging as a tool for mapping fibro-fatty lipid substrate.

5.4 DISCUSSION

5.4.1 Major Findings

The present study provides new mechanistic insights into the nature of fibro-fatty infiltrations as an evolving substrate for AF obesity and in fluctuating weight. Using a chronic ovine sheep model:

Compared to reference controls, atrial substrate due to chronic obesity was characterized by:

1. 13.8 mm Hg greater systolic blood pressure
2. 32% increased left atrial volume
3. 2-fold greater left and right atrial pressures
4. Significant fibrofatty infiltrations characterised by excessive presence of adipocytes, collagen and inflammatory infiltrates
5. 2.5-fold extensive myolysis and deterioration of myocyte contractile apparatus
6. Nonsignificant change in right atrial dimension

Atrial substrate due to weight fluctuation was characterised by:

1. Increased left atrial size compared to controls, but less severe compared to stable obesity
2. Nonsignificant change in right atrial volume compared to controls or to stable obesity
3. 1.5 times higher left atrial pressure compared to controls, but less severe than in obesity
4. Nonsignificant change in right atrial pressure compared to controls, but reduced in comparison to obesity
5. Significant fibrofatty infiltrations characterised by excessive adipocytes, collagen deposits and inflammatory infiltrates, but comparable to stable obesity
6. 2-fold significant and progression of myolysis of myocytes, but 30% less severe than in stable obesity
7. Characteristic profile and abundance of lipid species in the atrial myocardium
8. Nonsignificant change in systolic blood pressure

In three groups, atrial substrate is characterised by:

1. Significant negative correlation between mean grade infiltration and conduction velocity

2. Significant positive correlation between mean grade infiltration and electrogram fractionation
3. Significant correlation of grade infiltration with left atrial volume, but no correlation with right atrial volume
4. Nonsignificant correlations of infiltrations with left atrial voltage nor refractoriness
5. Significant correlations of myolysis with:
    a. Body weight
    b. Left atrial volume
    c. Left and right atrial pressures
6. Nonsignificant correlations between myolysis and systolic blood pressure and right atrial volume

In summary, weight fluctuation demonstrated an established pathological atrial remodelling despite comparable volumes of epicardial fat depots as lean weight and showed similar atrial substrate to obesity, albeit to a lesser extent.

5.4.2 Pro-arrhythmic Substrate Due to Obesity

Obesity is reported to associate with an increased risk of atrial arrhythmias in several epidemiological studies.[416, 421] Indeed, intensive research is underway to delineate the mechanisms that may mediate this sinister clinical link. Given the low-grade inflammation, neurohumoral activations, and autonomic imbalance seen during chronic obesity, it is likely that they might drive the formation of AF substrate in obesity.[181, 340] Consistent with this, in an earlier model of short-term obesity, we showed significant atrial enlargement, induction of fibrosis, and inflammatory infiltrates.[181] Obesity was associated with greater expressions of pro-fibrotic markers, including endothelin (ET)-1, ET receptors (ET_AR & ET_BR), transforming growth factor-beta 1 and platelet-derived growth factor in dose-dependent fashions. Mahajan et al.[182] corroborated these findings by demonstrating LA enlargement, increased interstitial fibrosis with accompanying pro-fibrotic TGF-β1 expression using a sustained model of obesity, induced by high-calorie feeding for 72 weeks. Interestingly, in the present study involving a very chronic model of obesity, we showed both abnormal atrial dimensions and haemodynamics.

Impairment in electrophysiological properties of the atrial is an important further requisite for AF substrate formation.[11] We previously reported global biatrial endocardial remodelling characterized by conduction abnormalities, fractionated electrograms, and increased propensity for AF during sustained weight gain, highlighting that obesity could induce electrical remodelling.[182] This is well in line with current findings. Nonetheless, unlike previous studies, we failed to association of obesity with reduced posterior LA endocardial voltage, which we speculate could be blunted by chronic age of our models.

5.4.3 Epicardial Fat and Pro-arrhythmic Substrate

Epicardial fat expansion is an important factor involved in the pro-arrhythmic substrate formation. In clinical reports, EAT is shown to predict greater risk for AF presence, severity and recurrence after ablation.[263, 265, 266] When compared to traditional markers of adiposity, such as BMI and waist circumference, EAT demonstrates superior risk prediction for AF. The link between EAT and AF has been further demonstrated several preclinical models. For example, experimental studies have shown that cardiac MRI assessed EAT expansion occurs as a common consequence of obesity and that it may explain the clinical link between obesity and AF.[181, 182] Additionally, EAT secretome is shown to promote fibrosis, alteration of pro-fibrotic signalling, myocyte electrical properties.

5.4.4 Epicardial Fat-mediated Ultrastructural Remodelling

According to the prevailing hypothesis, induction of fat cell infiltration could occur as a consequence of epicardial fat expansion, further adding to the substrate due to obesity.[182, 280, 388, 412] This was recently confirmed in an experimental study by Mahajan et al[182], which demonstrated significant invasion of contiguous posterior left atrial myocardial walls by epicardial fat cells following long-term weight gain. In the current study, we noted substantial presence of infiltrated intramyocardial fat cells. When we studied this a bit more in-depth, we discovered that these fatty infiltrates were more extensive and severe in chronic obesity and weight fluctuation, which were characterised by increased presence of fibrotic scar tissue and

inflammatory infiltrates. The adipocytes exhibited signs of cell fusion more characteristics of syncytia formation rather than hypertrophic growth.

Myocardial fibro-fatty infiltrations represent more than just an epiphenomenon. The electrical inertness or lack of heat conductivity of fat is a long-established concept[281, 422] and could, indeed, underlie sinister pro-fibrillatory mechanisms.[11, 120] Consistent with this, our data showed significant correlation of fibro-fatty infiltrates with complex electrogram fractionations, which serve as cardinal signs of complexity in fibrillatory circuitry.[11] We propose that these non-excitable cells could promote reduction in cell–cell coupling and increased local conduction blocks that can promote re-entry, electrical dissociation, and wave breakthrough.

Furthermore, the presence of fatty infiltrates in the microenvironment of myocytes may aggravate the paracrine effects of the EAT secretome. This could permit a more direct modulating effect on the cardiac myocytes by secreting myriad cytokines with ability to alter the functional and structural properties of the atrial myocytes.[11, 120, 282] It is intriguing that, in the present study, our results demonstrated extensive myofibrillar remodelling, highlighting that increased adiposity leads to ultrastructural changes in the myocytes. Additionally, the extent of myofibrillar remodelling was correlated with atrial enlargement and haemodynamic impairments. This was observed in both the obese and weight fluctuation models, thus implying that fluctuating could promote ultrastructural change akin to obesity. It is likely that subcellular changes like myolysis and perturbation of lipid signature occur early on before overt AF substrate. This concept is well supported by other reports, which have shown that changes at subcellular levels, occurring early on in the time of structural remodelling (1 to 3 weeks), contribute to AF pathogenesis. Additionally, it could allow for closer and direct cellular crosstalk between fat cells and intramyocardial fibroblasts, increasing the likelihood of the latter to transmogrify into aggressive collagen-depositing myofibroblasts.[388] It is intriguing that the results reported herein demonstrated extensive fibrotic scare formation with progressive severity of fibro-fatty infiltrates.

5.4.5 Atrial Substrate in Weight Fluctuation

Obesity relapse remains one major bottleneck in weight management programs. Data from several trials have implicated compensatory mechanisms, such as reduced energy expenditure, changes in adipogenic (i.e., leptin) and appetite regulating hormones, as likely culprits.[343, 344, 419] Potential cardiomodulatory effect of weight fluctuation was recently demonstrated by us. In a long-term follow-up study, we showed worsening of benefits of weight loss with more than 5% fluctuation in weight of patients.[345] We speculate that the changing adipokines following fluctuating weight may modulate atrial electrophysiology and predispose to AF. Indeed, recent data suggest that leptin may mediate angiotensin-II-[423] and high-fat-diet-induced[424] atrial fibrosis and induction of AF. It is notable that, in the present study, demonstrated residual areas of atrial enlargement and atrial pressure increases, which could mediate diastolic dysfunction and AF substrate. More importantly, we noted significant fibrofatty infiltrations and myolysis, which correlated strongly with structural and haemodynamic remodelling during in weight fluctuation. We believe this data may plausible explanation to the results of the LEGACY study. Taken together, it is possible that stable obesity and WF promote atrial remodelling via alternate upstream mechanisms, the EAT-trigger and compensatory adipokine (EAT-independent) pathways, respectively.

5.4.6 Study Limitations

Several limitations are worth noting in the current study. First, there was no segmentation performed for atrial EAT due to the limited resolution of CMR. We believe that this could have hidden important details on the regional distribution of EAT, which may have prevented conclusion on regions that may of important clinical relevance. Segmentation of EAT, as reported by Nagashima et al[302], would have allowed us to determine EAT location that most likely mediate the observed elecro-structural substrate seen therein. It should be noted that those investigators used computed tomography as their imaging modality, which demonstrates much higher resolution.

5.4.7 Potential Clinical Implications

These findings provide a mechanistic basis for the clinical associations reported between expansion of epicardial fat and AF. EAT may represent a useful risk marker to identify patients at an increased AF risk which could allow a more personalized risk stratification. EAT could be a promising target for atrial fibrillation, a concept recently tested in patients who underwent pulmonary vein isolation wherein treatment with artovastatin led to reduction in EAT volume. Furthermore, these results provide mechanistic basis for the perils of periodic weight fluctuation often encountered in clinical settings. Although weight loss is likely to reduce EAT, fluxes in weight could remodel patients' atria by induction of fibro-fatty infiltrations and pathologic modulation of contractile units in myocytes. Further studies are warranted to explore potential therapies targeting intramyocardial fat cell depositions and to determine whether their modulation could constitute a treatment target for primary and secondary prevention of AF.

5.5 CONCLUSIONS

The findings herein demonstrate that chronic obesity induces fibro-fatty replacement of atrial myocytes and deterioration of myocyte contractile apparatus, which may drive impairments of atrial electrical properties. Weight fluctuation induces similar but less severe changes to those seen during stable obesity and this may explain the increased risk of atrial arrhythmias during periodic fluxes in weight. Fibro-fatty infiltrations underlie important substrate for the pathogenesis of atrial fibrillation, which necessitate therapies targeting intramyocardial fat cell depositions.

5.6 TABLES

Table 1. Anthropometric and Haemodynamic Characteristics

Parameter	Control	Obese	Weight fluctuation	p-value		
				Obese vs. controls	Weight fluctuation vs. controls	Obese vs. weight fluctuation
Weight (kg)	76.1±4.5	109.1±7.1	77.2±4.5	<0.001	0.960	<0.001
Systolic BP (mmHg)	79.4±5.0	93.2±14.3	80.8±10.1	0.034	NS	0.053
LA pressure (mmHg)	3.7±0.9	8.5±1.9	5.7±1.8	<0.001	0.02	0.009
RA pressure (mmHg)	2.9±0.9	7.2±2.5	4.6±2.7	<0.002	NS	0.048
LA-EDv (mL)	34.2±5.2	43.3±6.6	33.0±6.6	0.239	0.884	0.086
LA-ESv (mL)	23.5±3.9	28.8±3.6	23.5±1.4	0.687	0.773	0.28
RA-EDv (mL)	34.1±4.8	47.9±5.3	35.0±13.2	0.247	0.962	0.318
RA-ESv (mL)	22.3±4.1	32.8±4.2	25.1±9.1	0.462	0.972	0.551
LV ejection fraction (%)	41.6 (8.7)	44.9 (8.3)	45.1 (10.2)	0.710	0.336	0.694

Table 2. Structural, Fibro-fatty Infiltrations, Myofibrillar Remodelling of Obese, Weight Fluctuation and Controls

Parameter	Control	Obese	Weight fluctuation	p-value		
				Obese vs. controls	Weight fluctuation vs. controls	Obese vs. weight fluctuation
LA volume (cm^3)	40.6±3.0	53.6±5.8	48.5±6.6	0.001	0.025	<0.001
RA volume (cm^3)	71.5±12.2	74.1±7.0	63.7±7.6	0.841	0.224	0.067
Fibrofatty infiltration grade, LA	1.54±0.29	2.51±0.41	2.35±0.42	0.001	0.004	0.710
Fibrofatty infiltration grade, RA	1.81±0.25	2.85±0.44	2.85±0.44	0.002	0.047	0.249
Total myolysis, LA (%)	38.3±16.3	95.8±2.0	70.2±9.3	<0.001	<0.001	0.012
Total myolysis, RA (%)	40.0±19.6	92.3±10.1	82.5±11.0	0.001	0.001	0.920
Severe myolysis, LA (%)	5.7±4.9	42.7±5.1	17.2±9.5	<0.001	0.042	<0.001
Severe myolysis, RA (%)	1.6 (8.1)	34.6 (40)	24.9 (24.2)	0.02	0.006	0.671

Table 3. Relationship Between Mean Grade Fibrofatty Infiltration and Electro-structural Substrates

Parameter	Pearson *r*	r^2	*p*-value
LA pressure (mm Hg)	0.620	0.384	0.005
RA pressure (mm Hg)	0.315	0.099	0.175
LA voltage (mV)	-0.076	0.006	0.756
CV, LA (m/s)	-0.592	0.350	0.006
CV, RA (m/s)	-0.721	0.520	0.001
ERP mean, LA (ms)	-0.102	0.010	0.679
ERP mean, RA (ms)	-0.078	0.006	0.749
LA fractionation (mm)	0.706	0.498	<0.001

Table 4. Relationship Between Mean Atrial Myolysis and Atrial Structural Substrates

Parameter	Pearson *r*	r^2	*p*-value
Body weight (kg)	0.540	0.392	0.025
Systolic BP (mm Hg)	0.443	0.196	0.07
LA volume (cm^3)	0.612	0.374	0.009
RA volume (cm^3)	-0.308	0.095	0.228
LA pressure (mm Hg)	0.633	0.40	0.008
RA pressure (mm Hg)	0.532	0.283	0.028
LVEF (%)	0.237	0.056	0.394

5.7 FIGURE LEGENDS

Figure 1. Scoring Algorithm for Classifying Fibrofatty Infiltrations

Figure 2. Changes in Atrial Pressure

Panel A: Left atrial pressure demonstrating significantly increased diastolic and systolic pressure measurements caused by stable obesity and weight fluctuation. **Panel B:** Right atrial pressure demonstrating significant diastolic and systolic pressure changes due to obesity; chamber pressure increased caused by weight fluctuation trended towards significance. **CN**, controls; **OB**, obese group; **WF**, weight fluctuation group.

Figure 3. Fibro-fatty Remodelling of the Atrial Myocardium due to Obesity and Weight Fluctuation

Panel A: Representative Masson's Trichrome-stained images of the atrial myocardial tissue of control, obese and weight fluctuation animals (**x0.6 mag, 2.5 mm**); inset, showing fibrotic remodelling (arrowheads) of infiltrates in obese and weight fluctuation (**x20 mag, 100 µm**). **Panel B:** Characteristic hyperplastic nature of fibro-fatty infiltrations in obese and weight fluctuation animals; arrowheads pointing to inflammatory cells in the infiltrates (**x20 mag, 100 µm**). **Panel C:** Demonstrations of fat cell fusions in obese and weight fluctuation animals.; arrowheads pointing to giant syncytiated adipocytes. **CN**, controls; **OB**, obese; **WF**, weight fluctuation; **mag**, magnification

Figure 4. Characterisation of Fibro-fatty Infiltrations

Panel A: Average fibro-fatty infiltration grades of the left and right atrial tissues from controls, obese and weight fluctuation sheep. **Panel B:** Association of obesity and weight fluctuation with progressive severity in fibro-fatty infiltrations. **Panel C:** Collagen content of

infiltrates. **Panel D:** Average number of adipocytes per infiltrate. **LA,** left atrial wall; and **RA,** right atrial wall.

Figure 5. Relationship Between Fibrofatty Infiltration and Effective Refractory Period

Panel A: Linear regression results demonstrating no correlation between grade infiltration and ERP in the left atrium. **Panel B:** Correlations between grade infiltration and ERP in the left atrium. **ERP,** effective refractory period; **LA,** left atrial; **RA,** right atrial.

Figure 6. Relationship Between Fibrofatty Infiltration and Conduction Velocity

Panel A: Linear regression results demonstrating negative correlation between grade infiltration and CV in the left atrium. **Panel B:** Strong positive correlations between grade infiltration and conduction velocity in the right atrium. **CV,** conduction velocity; **LA,** left atrial; **RA,** right atrial.

Figure 7. Relationship Between Fibrofatty Infiltration and Fractionated Electrogram and Voltage

Panel A: Linear regression results demonstrating strong positive correlation between grade infiltration and electrogram fractionation in the left atrium. **Panel B:** No correlation between grade infiltration and voltage in the left atrium. **LA,** left atrial; **RA,** right atrial.

Figure 8. Remodelling of the Myofibrillar Contractile Units

Panel A: Representative periodic acid Schiff stained images, demonstrating replacement of the myofibrillar units (myolysis) of atrial myocytes (**x80 mag, 25 µm**). **Panel B:** Percent total myolysis in controls, obese and weight fluctuation groups in the left and right atrial chambers. **CN**, controls; **LA**, left atrial; **OB**, obese; **RA**, right atrial; **WF**, weight fluctuation.

Figure 9. Progressive Remodelling of Myofibrillar Contractile Units

Progressive severity of myolysis because of stable obesity and weight fluctuation, proportion of mocytes with no myolysis (**Panel A**); mild myolysis (**Panel C**); moderate myolysis (**Panel**

C); and severe myolysis (**Panel D**). **CN**, controls; LA, left atrial; **OB**, obese; **RA**, right atrial; **WF**, weight fluctuation.

Figure 10. Relationship Between Myofibrillar Remodelling and Systolic Blood Pressure

Panel A: Linear regression results showing non-significant weak correlation of myolysis in the left atrium with systolic blood pressure. **Panel B:** Regression results showing weak and non-significant correlation of myolysis in the right atrium and systolic blood pressure. **BP**, blood pressure; **LA**, left atrial; **RA**, right atrial.

Figure 11. Relationship Between Myofibrillar Remodelling and Chamber Volumes

Panel A: Linear regression results showing strong, significant positive correlation of myolysis with chamber volume in left atrium. **Panel B:** Regression results showing non-significant negative correlation with chamber volume in the right atrium. **BP**, blood pressure; **LA**, left atrial; **RA**, right atrial.

Figure 12. Relationship Between Myofibrillar Remodelling and Atrial Haemodynamics

Panel A: Linear regression results showing significant positive correlation of myolysis with pressure in the left atrium. **Panel B:** Regression results showing significant positive correlation with pressure in the right atrium. **LA**, left atrial; **RA**, right atrial.

Figure 13. Correlation of LV Ejection Fraction with Atrial Myofibrillar Remodelling

Panel A: Linear regression results showing no correlation of left atrial myolysis with left ventricular ejection fraction (x100). **Panel B:** Regression results showing no correlation of right atrial myolysis with left ventricular ejection fraction. **LA**, left atrial; **RA**, right atrial.

Figure 14. Spectral Distribution of Most Abundant Peaks of Matrix-assisted Laser Desorption Ionization Images

Panel A: Representative H & E staining images and orientation (epicardium to endocardium) of sheep posterior left atrial tissue showing distribution of fatty infiltrations. **Panel B:**

MALDI images of lipid with the most abundant peaks. The identified lipids are indicated at the top; *m/z* values are shown above the images. The intensity of each pixel reflects the abundance in the tissue.

Figure 1. Scoring Algorithm for Classifying Fibrofatty Infiltrations

A

Grade	Histological feature
1	Small area of adipocytes, limited or no fibrosis, no inflammation
2	Fat cell infiltration plus increased fibrosis, no inflammation
3	Increased infiltrated adipocytes plus increased fibrosis plus inflammatory infiltrates
4	Extensive infiltrated adipocytes, fibrotic scarring and inflammatory infiltrates

B

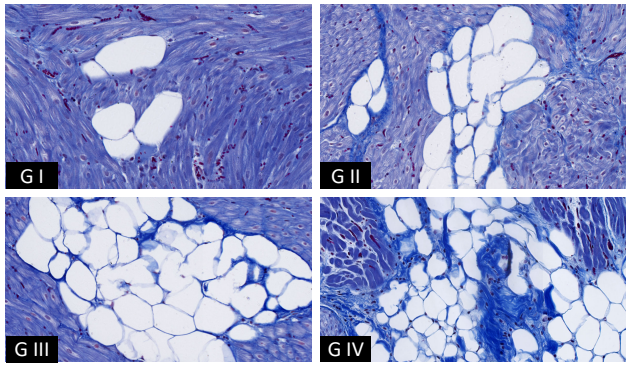


Figure 2. Changes in Atrial Pressure

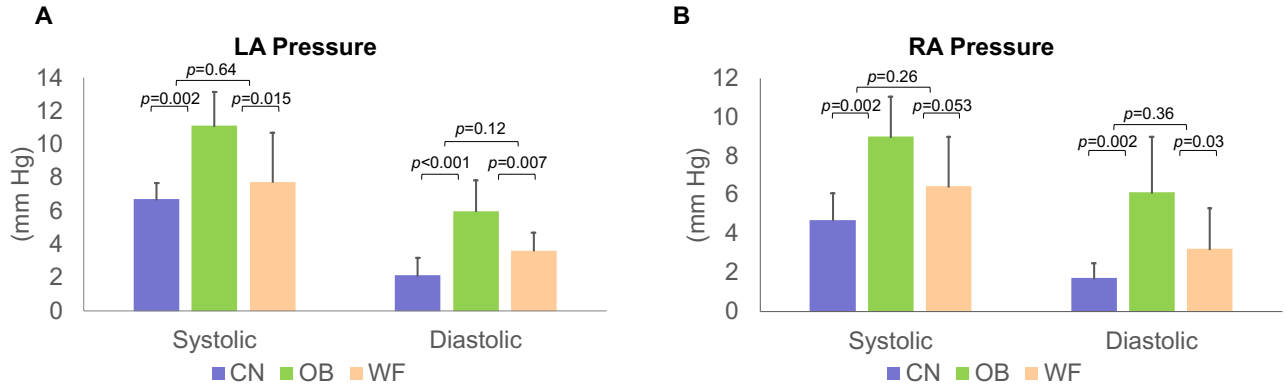


Figure 3. Fibro-fatty Remodelling of the Atrial Myocardium due to Obesity and Weight Fluctuation

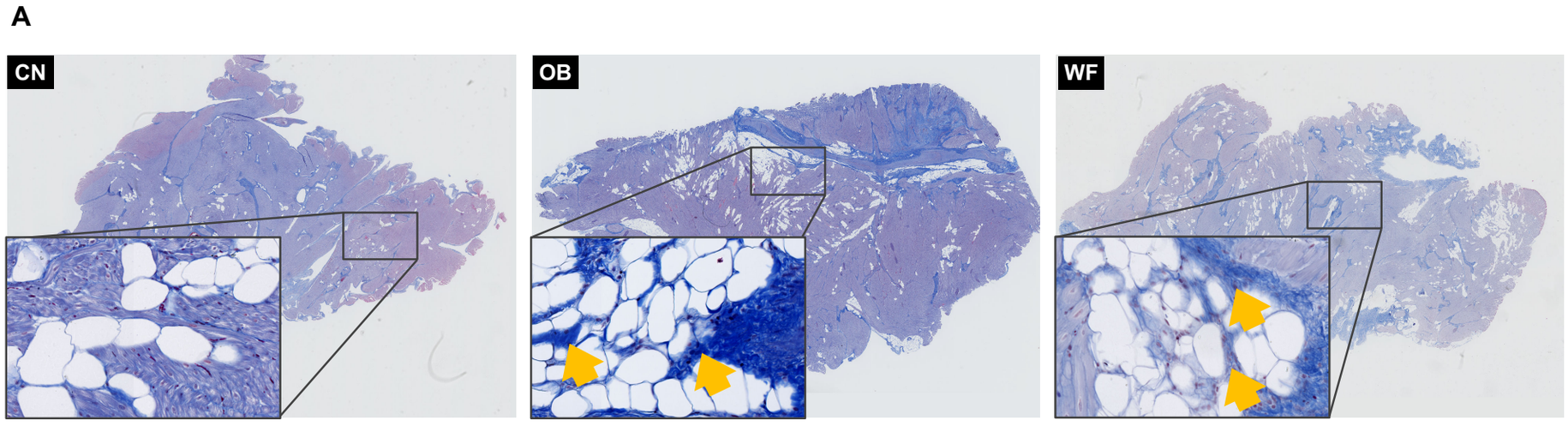


Figure 4. Characterisation of Fibro-fatty Infiltrations

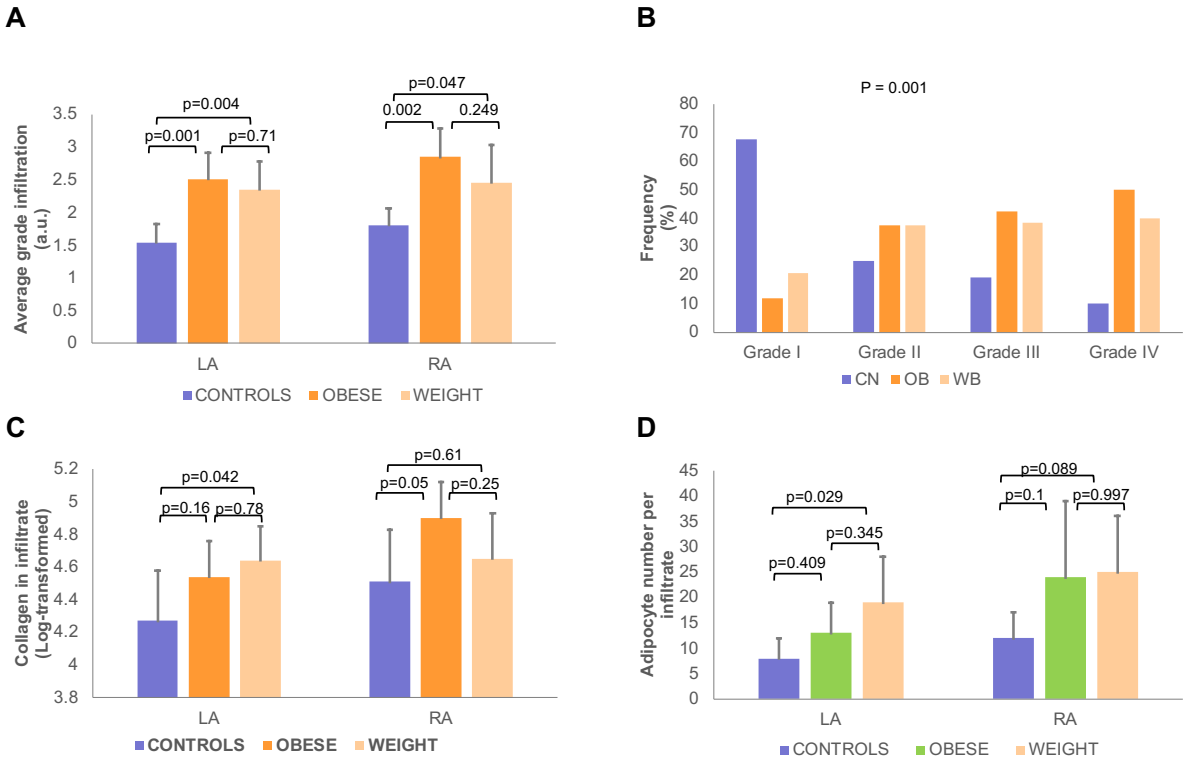


Figure 5. Relationship Between Fibrofatty Infiltration and Effective Refractory Period

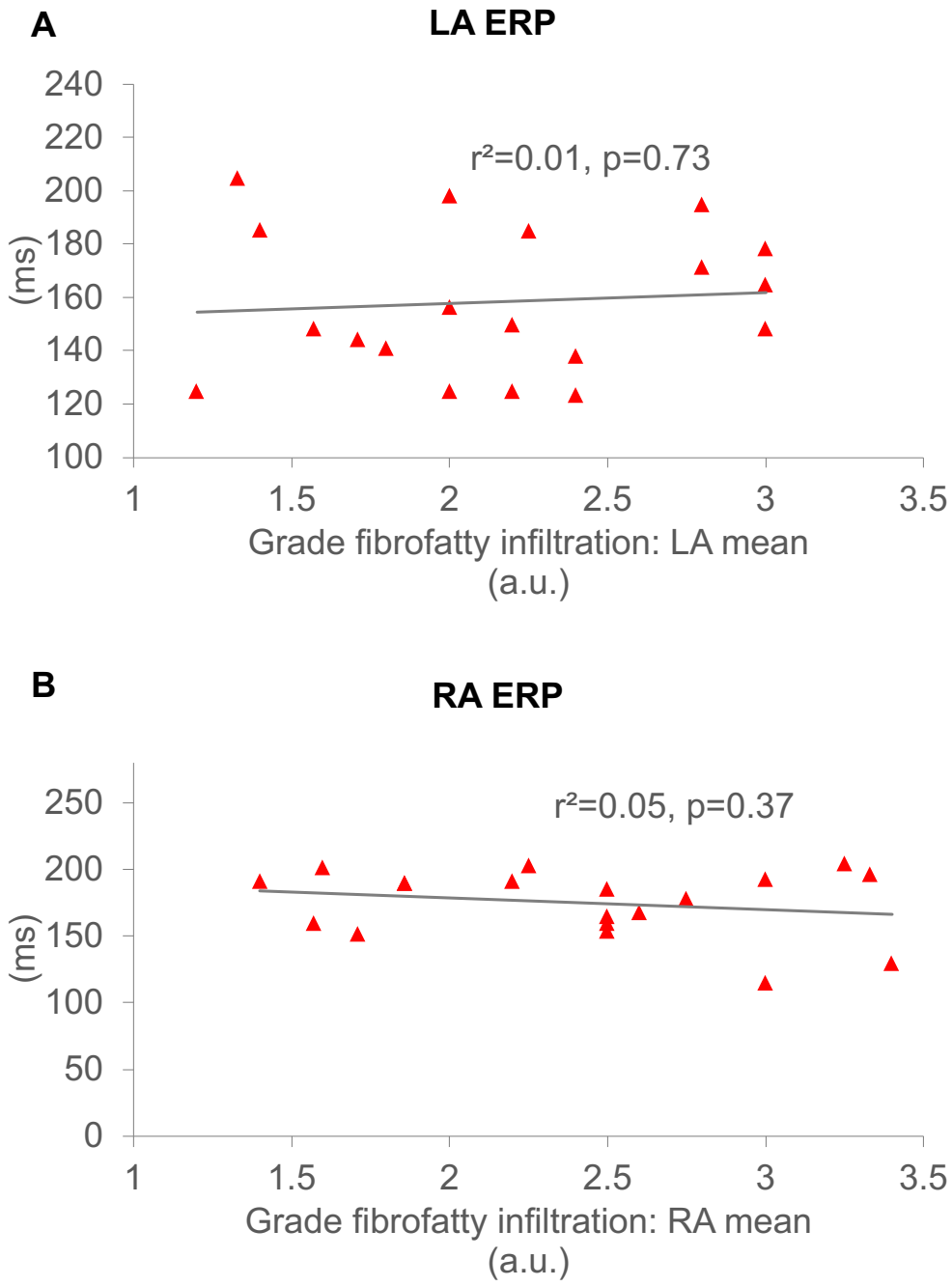


Figure 6. Relationship Between Fibrofatty Infiltration and Conduction Velocity

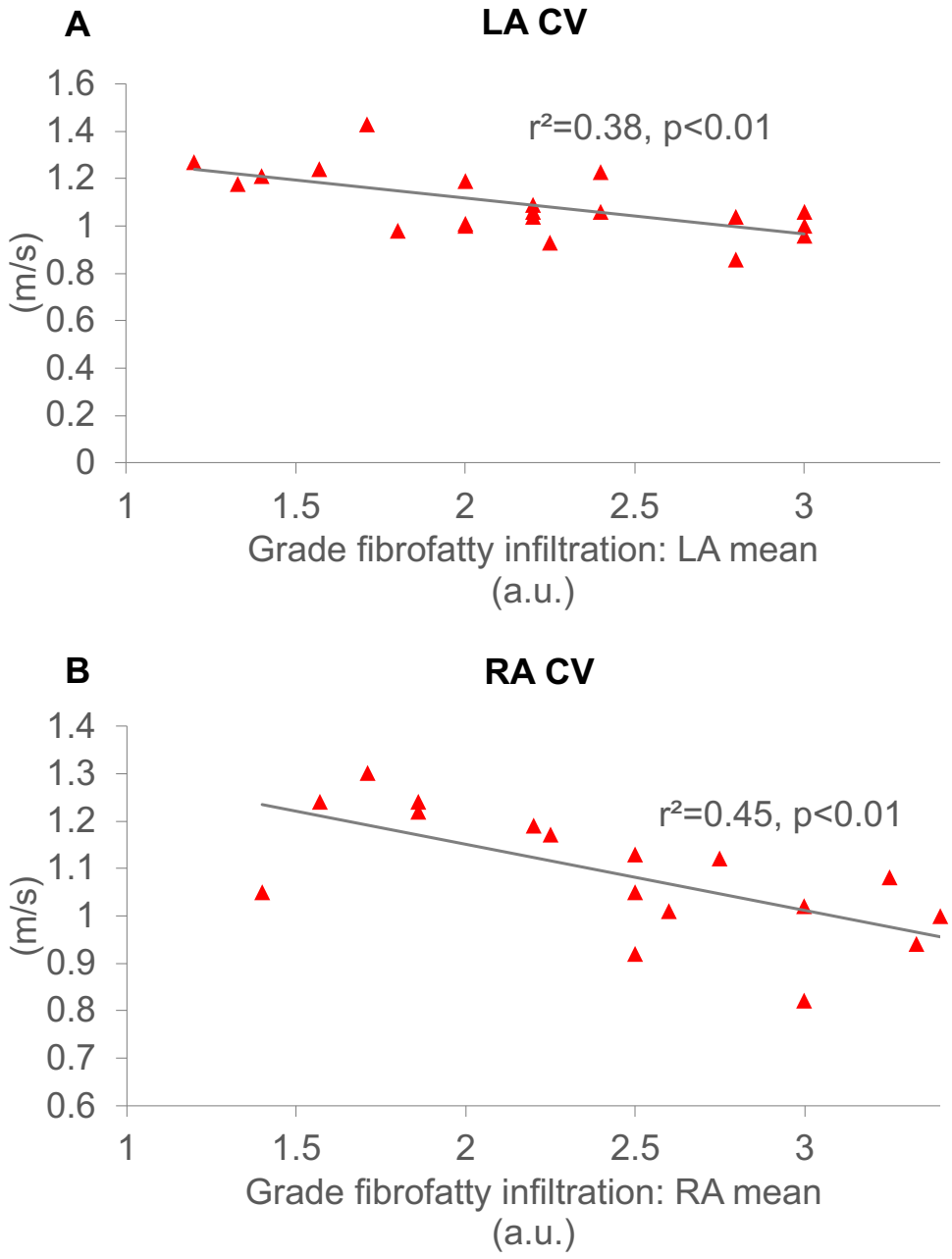


Figure 7. Relationship Between Fibrofatty Infiltration and Fractionated Electrogram and Voltage

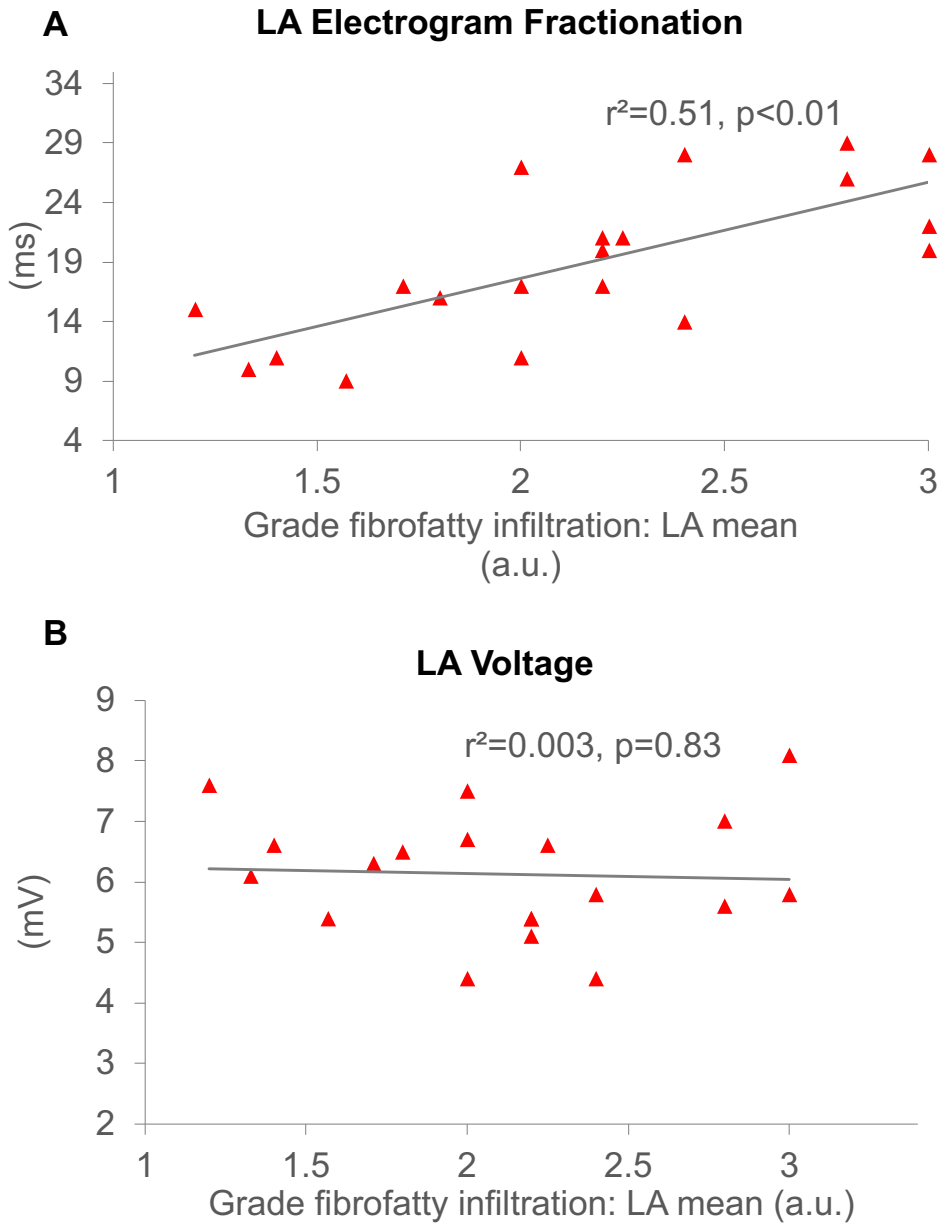


Figure 8. Remodelling of the Myofibrillar Contractile Units

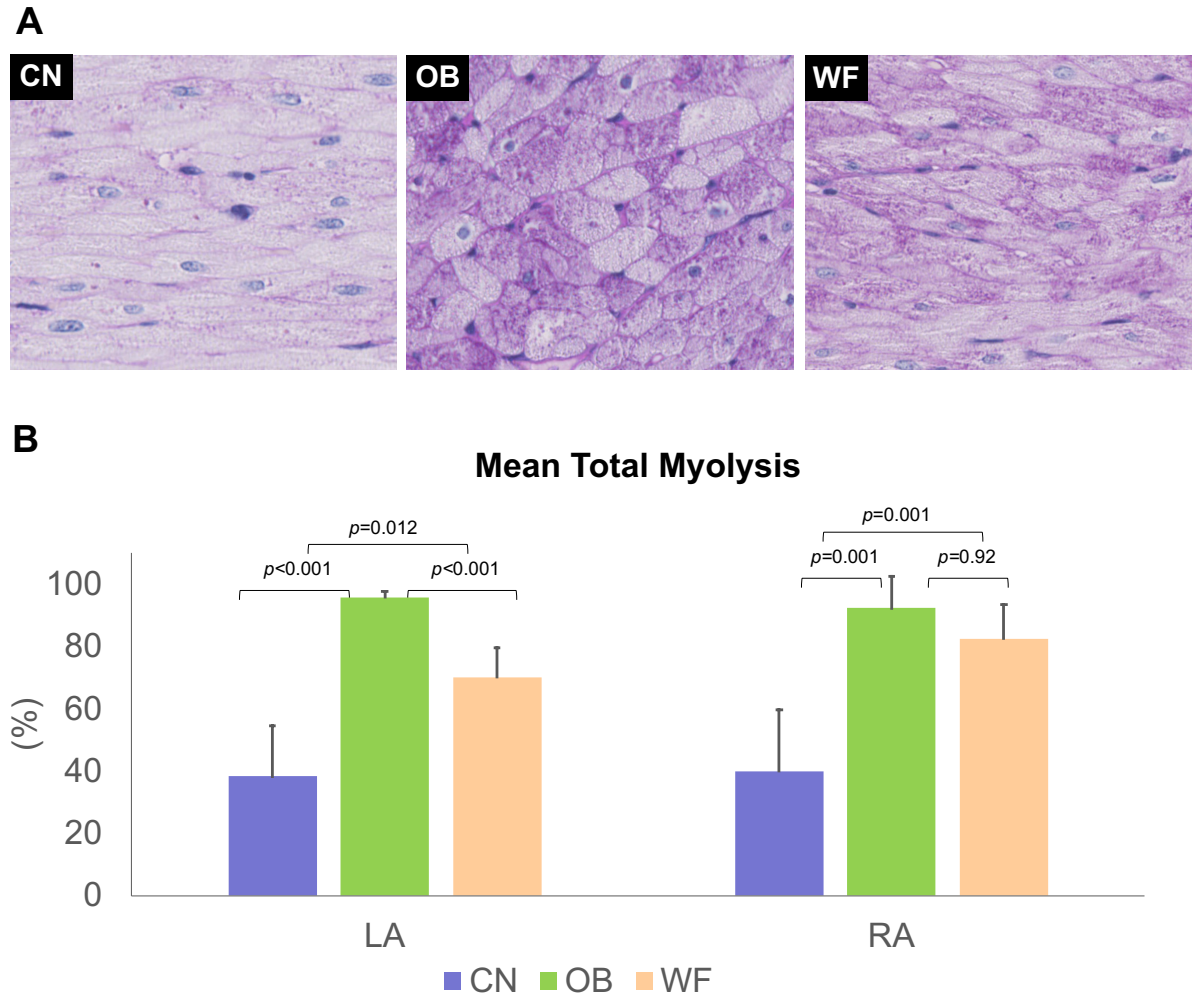


Figure 9. Progressive Remodelling of Myofibrillar Contractile Units

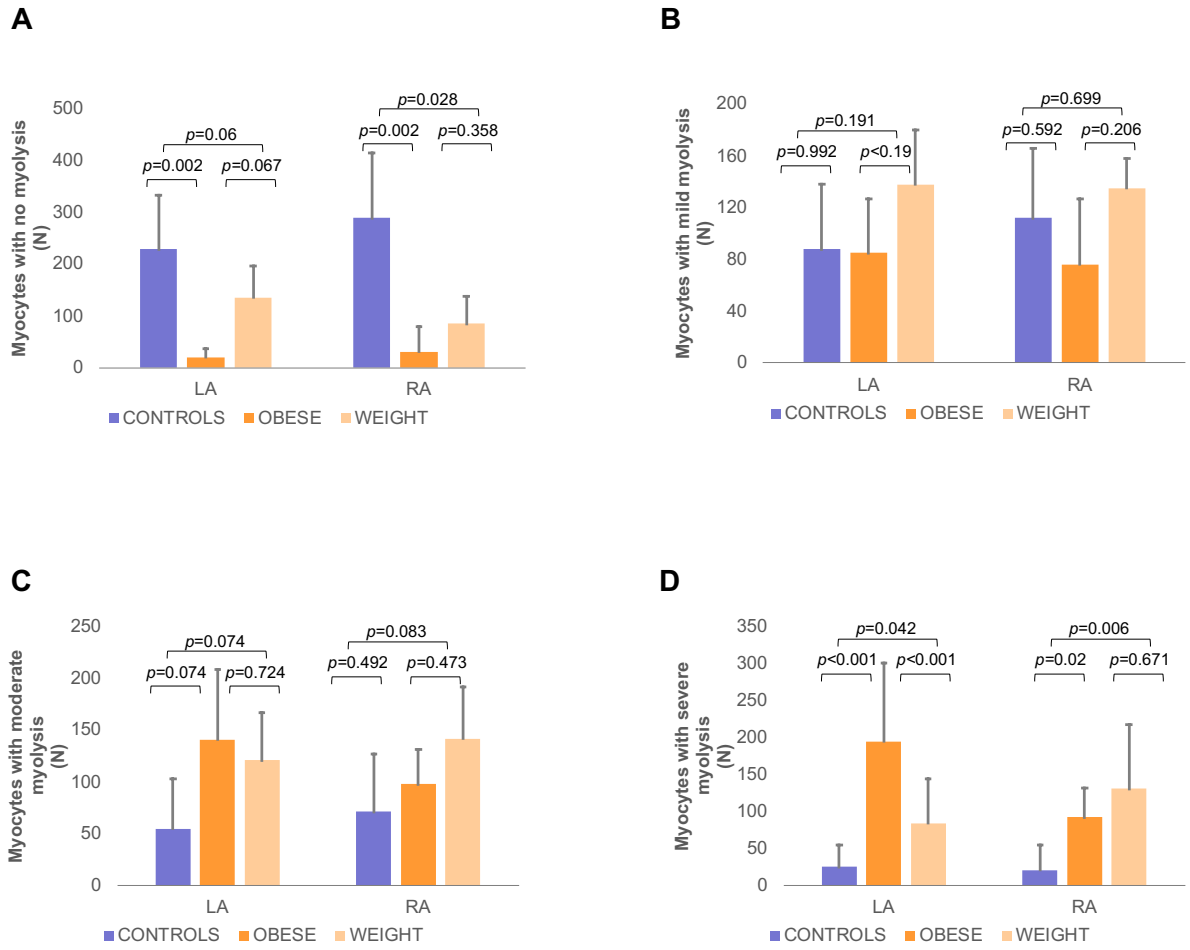


Figure 10. Relationship Between Myofibrillar Remodelling and Systolic Blood Pressure

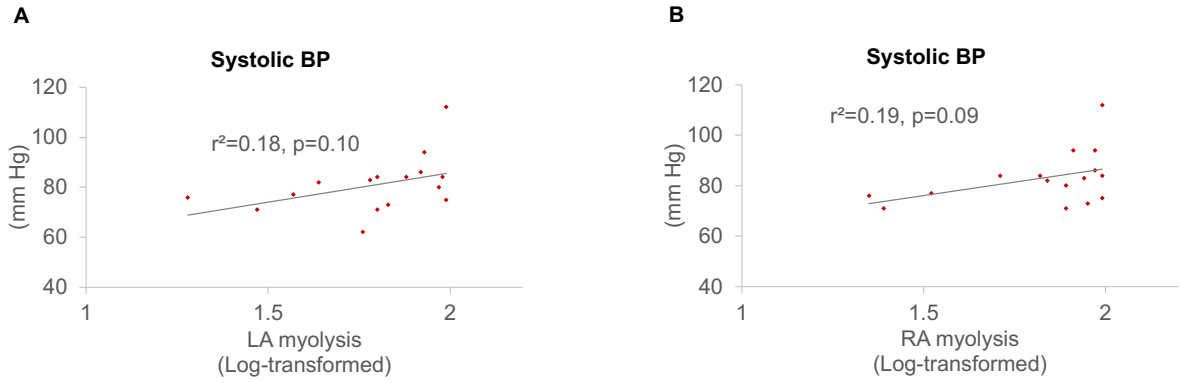


Figure 11. Relationship Between Myofibrillar Remodelling and Chamber Volumes

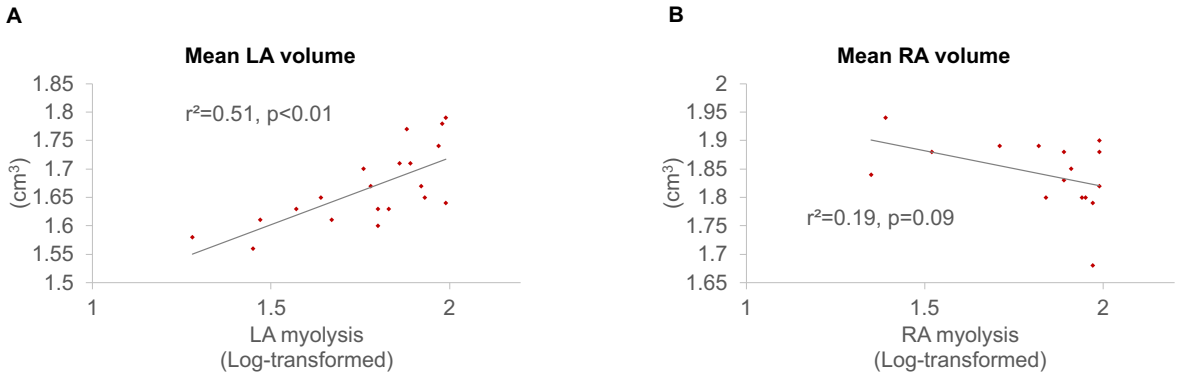


Figure 12. Relationship Between Myofibrillar Remodelling and Atrial Haemodynamics

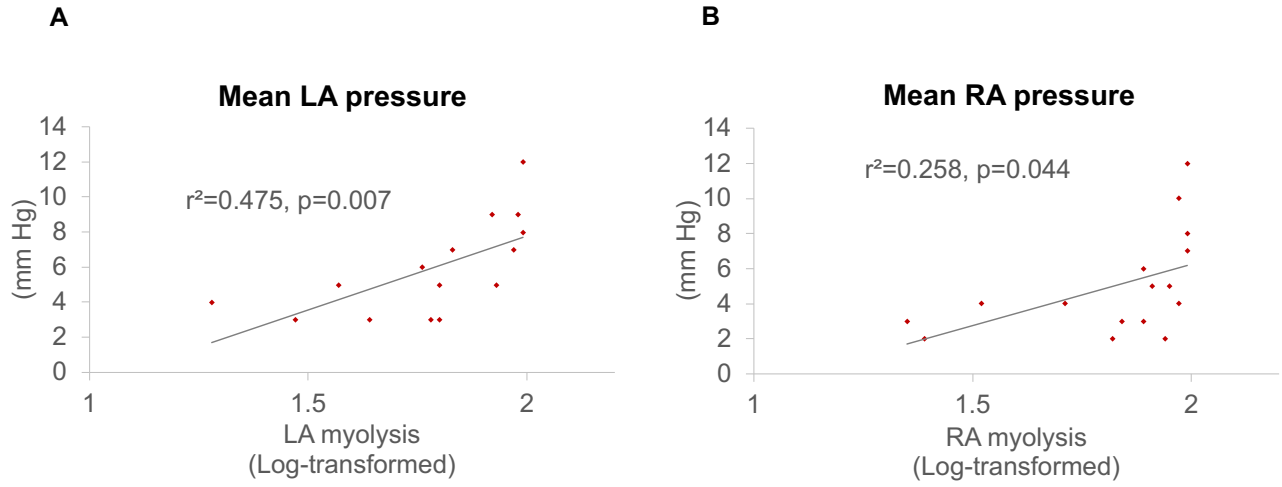


Figure 13. Correlation of LV Ejection Fraction with Atrial Myofibrillar Remodelling

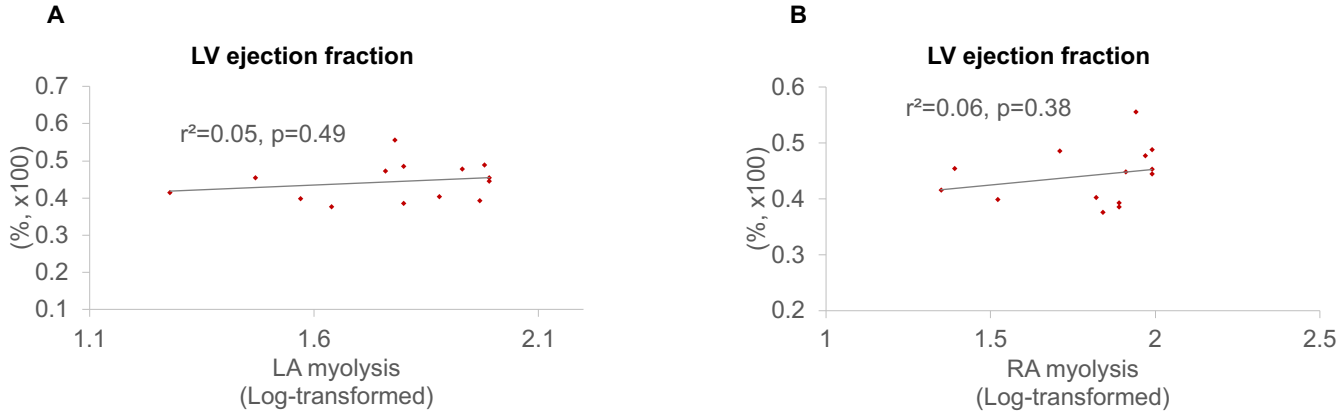
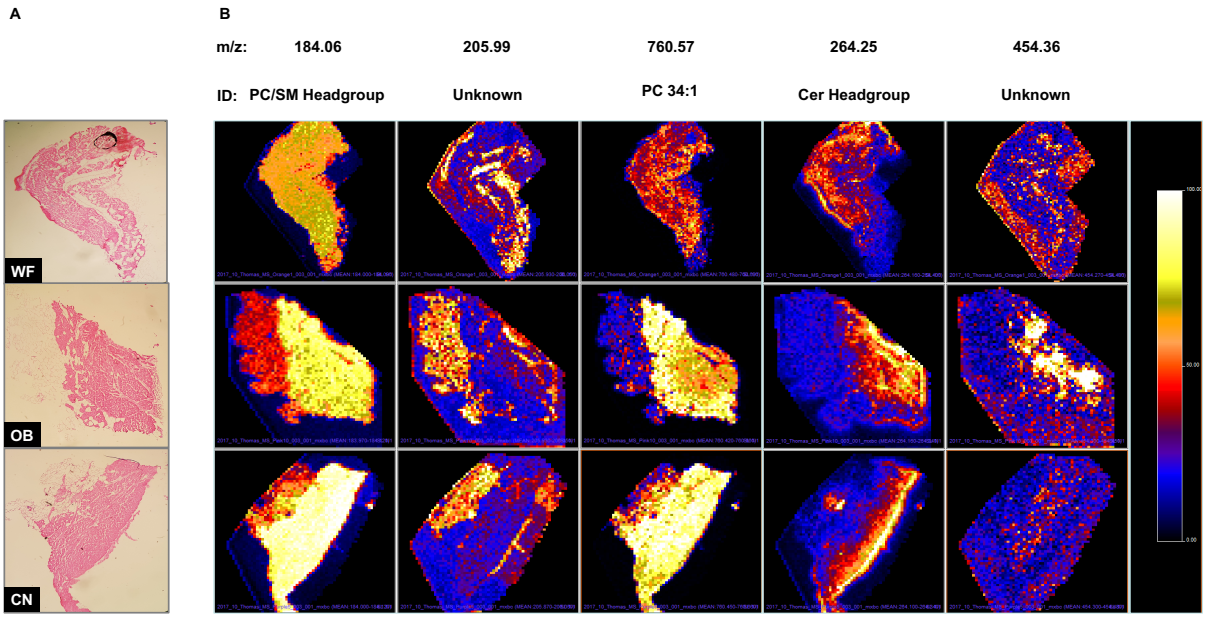


Figure 14. Spectral Distribution of Most Abundant Peaks of Matrix-assisted Laser Desorption Ionization Images



6. Chapter Six

Obesity and Sudden Cardiac Death: A Meta-Analysis of 1.4 Million Individuals

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6.1 INTRODUCTION

Sudden cardiac death (SCD) is responsible for 50% of total cardiovascular (CV) mortality and potential-life lost to CV disease.[307, 309, 425] According to recent epidemiological findings, while the overall burden of cardiac deaths has been gradually declining, the incidence of SCD has demonstrated a steady increase over the same period.[314, 426, 427] In the United States alone, SCD from out-of-hospital sudden cardiac arrest (OSCA) is reported to affect between 170,000 to 450,000, with incidence rates of 234 deaths per 100,000 person-years.[14, 309, 314, 426, 427]

Despite this, the true burden of SCD is likely to be inaccurately estimated due in part to the confusion in what constitutes sudden death. According to the most recent consensus documents, SCD is temporally defined as a natural death from "sudden" cardiac arrest (SCA) in patients without known cardiac abnormality, occurring within an hour of onset of symptom (witnessed) or 24 hours in an unwitnessed case; with cardiac arrest entailing the cessation of mechanical function or activity of the heart, evidenced by termination of both cardiopulmonary and systemic circulations.[309] Importantly, this takes into account three key elements of SCD: natural, rapid, and unexpected nature of the death.

Mechanistically, SCD is shown to be caused predominantly arrhythmic in origin[322], often precipitated by ischaemic heart disease[317, 318]. It is also precipitated by other pathological conditions, such as cardiomyopathies and inherited channelopathies.[312, 318] However, data from the general population demonstrate that half the crude rates of SCD occur in patients without apparent high-risk features or impaired heart function.[307, 331] Why this is the case remains unknown. Moreover, left ventricular dysfunction, the dominant

determinant for primary prevention implantable defibrillator (ICD), has been reported to only occur in the minority of SCD patients, thus highlighting the need for novel risk markers.[331]

The prevalence of overweight and obesity has more than doubled since the 1970's, with current projections estimating that up to 90% of middle-aged individuals will be either overweight or obese by 2025.[1, 3] Importantly, the rise of the obesity epidemic has culminated in rise of primary CV morbidities and primary risk factors for SCD.[1] Obesity, measured as body mass index (BMI), has been reported to show a graded and highly significant association with myocardial infarction in community-based studies.[335] There is also evidence suggesting that increasing adiposity may contribute to the pathogenesis of sudden death[337], with both moderate and severe obesity reported to associate with higher risk of pulseless ventricular tachyarrhythmia's and late potentials.[428, 429]

In this study, we aimed to undertake a systematic review of the literature and provide a meta-analytic assessment of the link between obesity and SCD. Our objectives were to evaluate the association between SCD and: 1) BMI as a categorical variable, and 2) BMI on a continuous scale in prospective observational studies.

6.2 METHODOLOGY

6.2.1 Literature Search Strategy and Selection Criteria

The present study was conducted in accordance with the guidelines given by the Meta-analysis of Observational Studies in Epidemiology (MOOSE) statement[430] and was registered on **PROSPERO (ID: CRD42018104848).**

We searched the medical literature using the online databases PubMed, EMBASE, Ovid MEDLINE, using the key words:

PubMed: (Overweight [MH] or Overweight [TW] OR Obesity [TW] OR Obesity [MH] OR body mass index [TW] OR BMI [TW]) AND (Sudden cardiac death [TW] OR Sudden Cardiac Death [MH] OR Sudden Cardiac Arrest [TW] OR Sudden Cardiac Arrest [MH] OR Sudden Arrhythmic Death [TW] OR SCD [TW] OR Out-of-Hospital Sudden Cardiac Arrest [TW] OR OSCA [TW])

EMBASE: ('Obesity'/EXP OR Obesity OR 'Obesity'/SYN OR 'BMI'/EXP OR 'BMI'/SYN OR 'Body Mass'/EXP OR 'BODY MASS'/SYN) AND ('Sudden Cardiac Death'/EXP OR 'Sudden Cardiac Death'/SYN OR 'Out of Hospital Cardiac Arrest'/EXP OR 'Out of Hospital Cardiac Arrest'/SYN OR 'Sudden Cardiac Arrest'/EXP OR 'Sudden Cardiac Arrest'/SYN)

The Core Collection of Web of Science: (Overweight OR Obesity OR "body mass index" OR BMI) AND ("Sudden Cardiac Death" OR "SCD" OR "Cardiac Arrest" OR "Out-of-Hospital Cardiac Arrest" OR "Sudden Cardiac Arrest" OR "OSCA" OR "SCA")

Ovide MEDLINE: (body mass index.mp. OR BMI.mp. OR Body Mass Index/ OR overweight.mp. OR Overweight/ OR obesity.mp. OR Obesity, Morbid/ OR Obesity/) AND (sudden cardiac arrest.mp. OR Death, Sudden, Cardiac/ OR SCD.mp. OR SCA.mp.)

Studies published in English were retrieved and exported to and sorted in EndNote 8.2 software. We screened the retrieved papers based upon the titles followed by the scrutiny of their abstracts and full texts, thereafter, and excluded: 1), conference reports and case reports; 2), abstracts not yet published, editorials and letters to the editor; 3), studies reporting only all-cause mortality and/or non-sudden cardiac mortality; 4), and studies that did not address study objectives. Review articles were searched for original papers and were later excluded. Finally, we included studies if they met the following criteria: 1), reported sudden cardiac death as an endpoint; 2), used BMI or WHR as the measure of obesity; and 3), conducted an adjusted multivariable risk estimation.

6.2.2 Data Extraction and Quality Assessment

Data extraction and study quality assessment were done by two investigators (Agbaedeng TA, TAA, and Munawar DA, DAM) independently, using an a priori determined set of guidelines, and with disagreements resolved by consensus. We extracted the following data: 1) The incidence of SCD/all-cause mortality/non-SCD; 2), Age; 3), Body mass index (BMI) and/or waist-to-hip ratio (WHR); 4), Risk estimates; 5), Follow-up; 6), Study endpoints; 7), Study design; 8), Participants. Methodological quality assessment was done via the "Newcastle-Ottawa Scale for Cohort Studies."

6.2.3 Data Analysis

A random effects meta-analysis was conducted on the pooled results from the various citations using the *RevMan 5.3 (The Cochrane Collaboration,Copenhagen)*, with the effect size presented as risk ratio or relative risk (RR). RRs were pooled from studies that

conducted multivariable analysis. Meta-analyses involving RR were done to show independent association with SCD.

Analysis was conducted on a categorical or continuous BMI, measured as kilogram per metre squared ($kg.m^{-2}$). BMI categories were defined as per World Health Organisation guidelines: <18.5 $kg.m^{-2}$ as underweight; 18.5 $kg.m^{-2}$ to 24.9 $kg.m^{-2}$ as normal weight; 25 $kg.m^{-2}$ to 29.9 $kg.m^{-2}$ as overweight; and ≥30 $kg.m^{-2}$ as obesity. The degree of heterogeneity of effect size estimates across the studies was assessed by examination of forest plots, chi-squared (X^2, or Chi^2) test and I-squared (I^2) statistic. The latter two provide numerical values for an assessment of heterogeneity, with a high Chi^2 relative to the degree freedom suggestive of variations in effect estimates and I^2 greater than 75% indicative of a considerable amount of heterogeneity ($p<0.1$ defined as the cut-off).

6.3 RESULTS

6.3.1 Literature Searching Results

Our online database searches on MEDLINE, Embase and the Cochrane Library resulted in a total of one hundred and ninety references, which were screened for eligibility. This was supplemented with hand-searching of the retrieved citations and searches done on sources like Google Scholar, thus, resulting in a total of 3925 references. Upon applying the exclusion and inclusion criteria (**Figure 1**), 22 studies were finally chosen for inclusion. Of these, five were further excluded for incomplete data reporting, leaving a total of 17 studies.

6.3.2 Study Characteristics

A full description of the characteristics of the included studies are provided in **Tables 1 and 2**, including study designs, quality scores, demographics, methodology, study endpoints, follow-up, and participants.

The 17 included studies had a total of 1,481,604 participants (586,641 [39.6%] males and 89,4963 [60.4%] females) from 5 countries (1 study from Canada[431], 5 from Finland[432-436], 3 from France[437-439], 2 from Japan[440, 441], 1 from Sweden[333] and 5 studies from the USA[337, 338, 442-444]). There were male- or female-only participants in 9 studies each. Three of the studies were multi-centre studies including individuals from multiple sites, including 2 studies performed in USA and 1 from France; there was no description of centres used in the rest of the studies. The follow-up period ranged from 8 years, in Albert et al[337] to as long as 56 years, in Cuddy et al[431] 27% of the studies had incident SCD/SCA as the primary outcome; 18.2% had both SCD and cardiovascular disease (CVD) or myocardial infarction (MI) as composite endpoints; 9% had coronary heart disease (CHD) as the endpoint; and in 45.5%, there was no indication that SCD was or not the primary outcome.

There was a total of 10,825 cardiac deaths reported in the studies, 8,151 (75%) of which were SCD, corresponding to an incidence rate of 5.50 deaths per 1,000 (8,151 SCD events/1,481,604 participants). SCD was adjudicated by a combination of review of medical records and autopsy reports, coroner, and by next-of-kin.

6.3.3 Study Quality

We assessed the methodological study quality using the Newcastle-Ottawa Quality Assessment Scale for cohort studies. Overall, the included studies earned NOS scores ranging from 5 to 9 (7.2±0.1), see **Tables 1 & 2.**

6.3.4 Clinical Characteristics

Patients diagnosed with SCD were older than event-free subjects (p=0.01) and had higher BMI (p<0.01), **Table 3**. Hypertension was the most prevalent condition in SCD subjects at 55%. SCD patients were 11 times more likely to have congestive heart failure than disease-free participants (p<0.01). Diabetes was prevalent among 22% SCD subjects compared to 4.4% in those without SCD (p<0.01). 24.1% and 16.0% of SCD diagnosed patients had a history of coronary heart disease and myocardial infarction, as compared to only 6.5% and 2.3% in SCD free patients (p<0.01 in all). Atrial fibrillation was also more common in SCD patients than in individuals without SCD ([6.3% vs. 3.9%, p<0.01], **Table 3**).

6.3.5 META-ANALYSIS

6.3.5.1 Incremental BMI and SCD

As shown in **Table 3**, BMI was significantly higher in patients with SCD than in those without sudden death. Seven studies including 50,552 individuals reported the association between increment in BMI and SCD risk.[333, 432-434, 437, 438, 440] Four population cohorts were reported in Lahtinen et al[433], thereby contributing a total of four risk estimates. The individual studies corrected for the following variables: alcohol, sex, cholesterol, age, systolic blood

pressure, prevalent CHD, smoking, diabetes, hypertension, left ventricular ejection fraction, triglycerides, fibrinogen, parental SCD, parental MI, and heart rate. In the pooled analysis, we found significant and independent association between BMI and SCD, such that 1-unit increase in BMI predicted more than 6% elevated risk of SCD (RR [95% CI]: 1.06 [1.02-1.10]; p=0.006), **Figure 2**.

6.3.5.2 Underweight BMI and SCD

Four studies reporting on underweight BMI and SCD in a total of 1,097,968 participants.[338, 436, 443, 444] Underweight BMI category was defined as BMI <18.5 $kg.m^{-2}$, except in Chiuve et al[338] and Eranti et al[436], where it was defined as 18.5 to 20.9 $kg.m^{-2}$ and <20 $kg.m^{-2}$, respectively. We pooled only the adjusted risk ratios from the individual studies in our analysis. Interestingly, an independent association of underweight with risk of SCD was only reported by Chiuve et al[338], but not shown for others. However, in our pooled analysis, this was significant and independent of traditional risk factors, such as: age, gender, smoking status, cholesterol, alcohol, family history of MI, diabetes, hypertension, prevalent CHD, prevalent HF, ECG variables. We found that the underweight predicted 33% increased risk of developing SCD (RR [95% CI]: 1.33 [1.00-1.78]; p=0.05), see **Figure 3**.

6.3.5.3 Overweight and SCD

We identified a total of 6 studies evaluating the relationship between the overweight state and the risk of SCD, and they had 1,381,477 participants.[337, 338, 435, 436, 442-444] Adabag et al[443] reported 2 sub-analyses, risk of SCD in individuals with and without a smoking history; therefore, we had a total of 7 risk estimates contributing to our meta-analysis. Overweight

was defined as BMI of 25 to 29.9 kg.m^{-2}. Other than in Chiuve et al[338] and Eranti et al[436], the rest of the studies did not show any significant association after adjusting for traditional risk factors. Accordingly, when we pooled these adjusted RR's in our meta-analysis, we found no significant association of the overweight BMI with SCD (RR: 1.13; 95% CI: 0.94 to 1.35; p=0.20), see **Figure 4**.

6.3.5.4 Obesity and SCD

We found a total of 10 studies providing data on the relationship of obesity and SCD, equating to 1,438,131 participants.[337, 338, 431, 435, 436, 439, 441-444] Given that Adabag et al[443] had two subgroup analyses, we had a total of 11 risk estimates that we pooled in our meta-analysis. After correcting for traditional risk factors, Adabag et al[443] (both in smokers & non-smokers), Bertoia et al[442] and Chiuve et al[338] did not find significant association between obesity and SCD. Overall, the included studies adjusted for: age, gender, smoking status, cholesterol, alcohol, family history of MI, diabetes, hypertension, prevalent CHD, prevalent HF, ECG variables. In our pooled analysis, obesity significantly associated with sudden death, predicting 44% elevated risk after correcting for covariates (RR: 1.44; 95% CI: 1.21 to 1.71; p<0.01), see **Figure 5**. Eranti et al[436] contributed the most to the weight of the estimates at 15.3%, with Chei et al[441] contributing the least at 1.2%. Given the unconventional definition of obesity by Empana et al[439] (BMI: 28.5 to 46.7 kg.m^{-2}), we excluded this study and still found a significant association (RR of 1.41 (95% CI: 1.18 to 1.68; p<0.01).

6.3.5.5 Waist-to-Hip Ratio and SCD

A total of 2 studies also looked at central adiposity measures as composite measures of obesity, including waist circumference and waist-to-hip ratio. Unfortunately, waist circumference was reported by only Bertoia et al[442], and WHR was grouped differently in the 2 studies that evaluated this, so no further analysis could be done.

6.3.6 Heterogeneity and Sensitivity Analysis

We evaluated statistical heterogeneity in the studies using Chi^2 and I^2 statistics. The pooled analysis for underweight BMI and SCD showed no evidence of inconsistency in effect size estimates (Chi^2: 2.04, df: 3, p=0.56; I^2: 0%), **Figure 3**. However, found moderate to substantial heterogeneity in the rest of the comparisons, see **Figures 2, 4 and 5**.

6.4 DISCUSSION

6.4.1 Major Findings

Obesity has been implicated in the risk of cardiovascular disease and is currently being investigated in the development of sudden cardiac death. In this meta-analysis, we explore the relationship between obesity and SCD, demonstrating that:

1. For every 1-SD increase in BMI significantly and independently associates with a 6% elevated risk of developing SCD, even after correcting for baseline covariates.
2. Underweight status (BMI <18.5 $kg.m^{-2}$) predicted 33% increased risk of SCD, independent of traditional risk factors.
3. Overweight (BMI 25 to 29.9 $kg.m^{-2}$) does not show significant association with SCD after baseline comorbidities are adjusted for, and.
4. Finally, obesity (BMI ≥30 $kg.m^{-2}$) was associated with higher risk of SCD. Even after correcting for traditional correlates, obesity still predicted 44% increased risk of the disease.

6.4.2 SCD and underweight: is undernutrition or excess weight loss to blame?

In the present study, we showed that underweight is associated with 33% greater risk of SCD compared to normal BMI. Indeed, our data is consistent with previously published reports. For example, Chiuve et al[338] demonstrated up to 58% increased risk of SCD for BMI <21 kg/m^2 in comparison with normal BMI. In another study involving first-time ICD recipients, underweight patients demonstrated ~2-fold greater odds of in-hospital death compared to

normal weight patients.[445] Taken together, these data show that low body weight may be detrimental in the context of SCD. It is notable that this comes at odds with the established benefits of weight loss for cardiac health. Moreover, the precise mechanism driving formation of SCD substrate in this cohort of patients remains evasive. Nevertheless, there are some data to suggest undernutrition or excessive weight loss may drive SCD risk modification.[446] Future research should aim to explore these questions in more detail.

6.4.3 Obesity and SCD

Sudden cardiac death is a major public health burden attributed to about 360,000 annual deaths (with estimates ranging from 180,000 individuals to as high as 450,0000 individuals) in the US alone, and a crude estimate at a staggering 5 million globally, making it the most significant cause of cardiovascular death worldwide.[14, 308, 309] More recently, high lifetime risk has been estimated for SCD, putting men at 10.9% and women at 2.8% at age of 45 years, and this is associated with higher aggregate burden of cardiac comorbidities.[314]

Obesity is strongly associated with increased prevalence of traditional risk modifiers of SCD. For example, in a prospective multicentre registry of individuals without CAD referred for coronary computed tomography angiography, higher BMI was positively associated with prevalence of any CAD and obstructive CAD.[332] Moreover, incident HF is overrepresented among obese individuals compared to people with normal BMI, and obesity was demonstrated recently as an independent predictor of HF with preserved ejection (HFpEF) as against HFrEF.[447] In the present meta-analysis, we show that obesity (defined as BMI ≥30 $kg.m^{-2}$) is associated with 44% increased risk of SCD, persistence even after correcting traditional risk factors, such as HF and CAD. It notable that, despite the obesity paradox

often reported in HF-related mortality[448], we did not observe this conundrum in the obesity and SCD relation. Furthermore, BMI on a categorical scale demonstrated a J-curve relation, with the best outcomes seen with normal weight and overweight status, respectively, see *Take home figure*. Our meta-analysis provides robust evidence for obesity and SCD risk. First, we provide a comprehensive evaluation of SCD risk in obesity. Second, the overall number of participants in this meta-analysis was very large. Third, the average follow-up period was long, ranging from at least 8 years to 56 years. Taken together, our data show that obesity may drive SCD independently of CAD/HF axis.

6.4.4 SCD Substrate in Obesity

Whether obesity is directly involved as a potential modifiable risk factor in SCD pathogenesis or a mere risk marker is not well described. Obesity has been correlated with changes in cardiac electrophysiological properties such as late potentials[429], signifying delayed activation in diseased myocardium and known marker for SCD, and shown to independently predict ventricular tachycardia/fibrillation (VT/VF)[428, 449].

The substrate for SCD in obesity is probably complex and multifactorial. Several structural and functional changes that have been described in obese hearts, such as: increased left ventricular diameters and mass, eccentric hypertrophy, diastolic dysfunction, and repolarisation abnormalities.[450-452] Moreover, QRS fragmentation (fQRS), representing heterogenous conduction and thereby fibrotic scars[453], is a common observation in obesity and obese patients dying from SCD[454]. Both fQRS and fibrosis are shown to predict SCD independently of reduced ejection fraction, highlighting the potential mechanism of ventricular remodelling in the obese with HFpEF.[455, 456]

Further, obesity is associated with volume overload and haemodynamic impairment, which may cause abnormal neurohumoral activations, leading to pro-fibrotic and inflammatory signalling.[457] Additionally, there may be several ways that obesity can lead to SCD. Obesity could impact SCD substrate by promoting traditional risk factors, coronary artery disease, and through direct cardiac effects.

6.4.5 Limitations

The amount of heterogeneity in some of the subgroup analyses is worth noting. Although this was significant, we believe that the level of heterogeneity, where it was found, was not critical, and may not have affected our risk estimates. The use of overwhelmingly non-randomised trials is an important drawback and may have introduced bias in the analyses. Notwithstanding this limitation, the methodology quality of these studies was moderately high, attesting to their internal validity. Finally, the disproportionately low number of males (37.3%) in the subjects is another limitation of our meta-analysis. It is well accepted that SCD has a male bias. In fact, the latest modelling study puts the life-time risk of SCD at 1 in 9 in males as compared to only 1 in 30 among female individuals.[314]

6.5 CONCLUSIONS

The present meta-analysis demonstrates an independent association between obesity and SCD. BMI is greatly increased in patients who die suddenly than non-SCD individuals, with 1-unit increment in BMI associated with greater risk for SCD. Further studies are warranted to delineate the mechanisms underlying this association and to explore the role of weight management in reducing the premature death due to SCD.

6.6 TABLES

Table 1. Characteristics of Studies Evaluating Obesity on a Continuous Body Mass Index Scale

Study ID	Country	Design	Risk Score	Follow-up Period	Study Endpoint	Ascertainment of SCD	Participants (% men)	Cardiac death (% SCD)	BMI ($kg.m^{-2}$)
Jouven et al. 1999	France	Prospective cohort	8	23 years	Hard CHD Major CHD event	Independent medical committee (ICD code 798.1)	7,079 (100)	603 (19.6)	25.4±3.3
Laukkanen et al. 2013	Finland	Prospective, population-based	8	18.8 years	ND	Independent events committee	2,641 (100)	190 (100)	26.9±3.5
Anderson et al. 2016	Sweeden	Prospective, nested case-control	7	20 years	CV events	Registry, discharge records and death certificates	2,361 (75.9)	363 (100)	27.5±1.1
Lahtinen et al. 2012	Finland	Population cohort	9	5 years	SCD	Independent physician reviews	27,629 (47.2)	494 (100)	26.7±0.4
Benchimol et al. 2000	France	Prospective	5	8.1±1.6 years	SCD & MI	ND	319 (85.9)	34 (74)	26.0±3.0
Laukkanen et al. 2009	Finland	Prospective, population-based	7	17.6 years	ND	Autopsy reports, interviews and Independent events committee	1,606 (100)	76 (100)	26.7±3.1
Kataoka et al. 2004	Japan	ND	7	6.5±4.8 years	SCD	Death certificates	8,917 (55.4)	56 (100)	23.6±3.2
TOTAL			**7.3±1.3**	**15.7±6.7**			**50,552 (62.1)**	**1,816 (72.8)**	**26.1±1.3**

BMI, body mass index; CHD; coronary heart disease; CV; cardiovascular; ICD, international classification of disease; MI, myocardial infarction; ND, not determined; SCD; sudden cardiac death.

Table 2. Characteristics of Studies Evaluating Obesity on a Categorical Body Mass Index Scale

Study ID	Country	Design	Risk Score	Follow-up Period	Study Endpoint	Ascertainment of SCD	Participants (% men)	Cardiac death (% SCD)	BMI Category
Cuddy et al. 2006	Canada	Prospective, longitudinal	6	56 years	ND	ND	3,983 (100)	171 (100)	Overweight Obesity
Eranti et al. 2016	Finland	Population cohort	9	35-41 year	ND	Review by 2 experienced cardiologists	10,543 (52.7%)	1,954 (39)	Lean Normal Overweight obese
Albert et al. 2003	United States	Prospective multiple-source surveillance	6	8 years	Incident SCA	Review by 2 cardiologists	121,701 (0)	2002 (100)	Underweight Normal Overweight Obese
Empana et al. 2004	France	Prospective cohort	8	>23 years	Incident	SCD	7079 (100)	603 (19.6)	Normal Overweight Obese
Chiuve et al. 2015	United States	Prospective	7	>32 years	Incident SCD	Next-of-kin Medical reports Autopsy reports	72,484 (0)	1,286 (34.6)	Lean Normal Overweight Class I obesity Class II obesity
Jae et al. 2018	Finland	Prospective, population cohort	8	22 years		Medical records	2357 (100)	253 (100)	Normal Overweight Obese
Chei et al. 2008	Japan	Prospective, population cohort	7	9.7±2.5 years	CHD	Medical records	43235 (100)	65 (100)	Normal Obese
Adabag et al. 2015	United States	Multicentre; Prospective cohort	7	12.6±2.5 years	ND	Next-of-kin Coroner Autopsy reports Committee of physicians	14,941 (45)	253 (100)	Lean Normal Overweight Obese Class II obesity
Bertoia et al. 2012	United States	Multi-centre Mixed: 3 RCT's & observational	6	10.8±2.8 years	Incident SCD	Witness interview Medical records	161,808 (0)	418 (100)	Underweight Normal Obese
Noheria et al. 2013	United States	Prospective Multiple-source surveillance	7	8 years	ND	Medical records autopsy	~1,000,000 (49)	2,004 (100)	Underweight Normal Overweight Obese
Total			**7.1±1.0**	**21.9±16.8**			**1,431,052 (38.8)**	**9,009 (75.8)**	

Table 3. Summary Baseline Clinical Characteristics

Co-variate	Incident SCD	SCD Free	Effects Estimate (95% CI)	p-value
Age, year	57.7±9.2	55.7±8.6	1.94 [0.38-3.50]	0.01
Diabetes	22.05%	4.4%	2.39 [1.46-3.90]	<0.001
Hypertension	55.06%	25.7%	1.99 [1.86-2.14]	<0.001
Body mass index, $kg.m^{-2}$	28.3±1.1	27.2±1.2	1.22 [0.88-1.56]	<0.001
Myocardial infarction	16.9%	2.3%	5.37 [3.08-9.35]	<0.001
Congestive heart failure	14.3%	1.3%	3.89 [2.03-7.44]	<0.001
Coronary heart disease	24.1%	6.5%	4.95 [2.32-10.57]	<0.001
Atrial fibrillation	6.3%	3.9%	2.58 [1.99-3.36]	<0.001

6.7 FIGURE LEGEND

Figure 1. A CONSORT Diagram of the Search Methodology

Figure 2. Risk of Sudden Death with Incremental BMI

This analysis evaluates the risk of SCD per 1-unit increase in body mass index (BMI) by pooling the multivariate adjusted risk ratios (RR) from the included studies. Adjustment was made for: hypertension; type diabetes; coronary heart disease; myocardial infarction; atrial fibrillation; sex; age; obstructive sleep apnoea; heart failure; and valvular heart disease. BMI was measured in kilogram per square metre ($kg.m^{-2}$).

Figure 3. Risk of Sudden Death in Underweight Subjects

This analysis evaluates the association of underweight BMI and the risk of sudden cardiac death (SCD). Please refer to figure 2 for factors adjusted for in the included studies. Underweight BMI was categorised as <18.5 $kg.m^{-2}$ or <20 $kg.m^{-2}$.

Figure 4. Risk of Sudden Death in the Overweight Subjects.

This analysis evaluates the association of overweight BMI and the risk of SCD. overweight BMI was defined as 25-29.9 $kg.m^{-2}$. Please refer to figure 2 for factors adjusted for.

Figure 5. Risk of Sudden Death in the Obese Subjects.

This analysis looks at the association of obesity with the risk of SCD. Obese BMI was defined as ≥ 30 $kg.m^{-2}$.

Take home figure. Schematic of SCD risks in different weight subclasses

The top schema shows the progressive development of substrates for ventricular arrhythmias and SCD. Bottom chart shows J-curve relation between BMI subgroups and the risk of AF, with the best outcomes seen in the normal weight and overweight groups, respectively.

Figure 1. A CONSORT Diagram of the Search Methodology

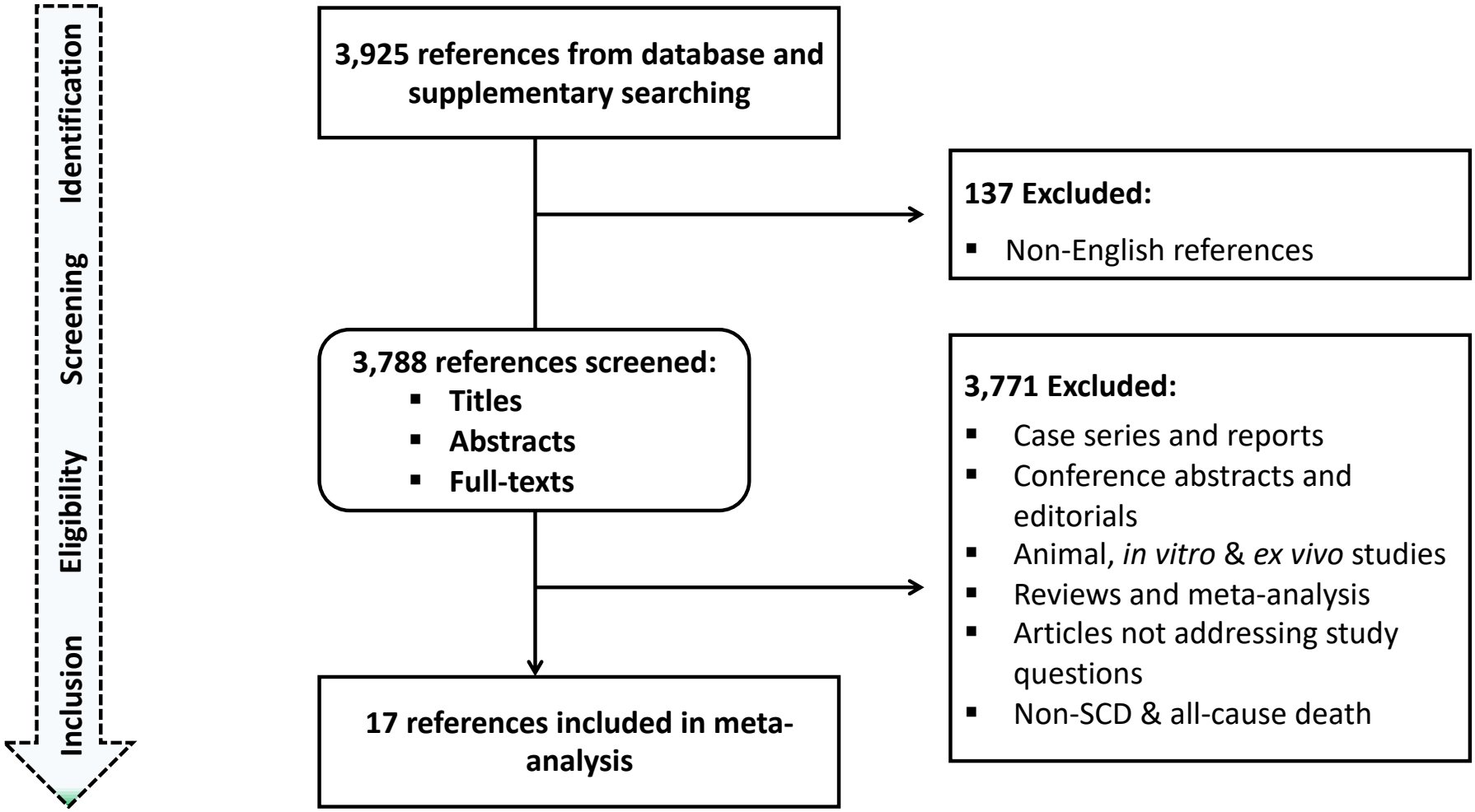


Figure 2. Risk of Sudden Death with Incremental BMI.

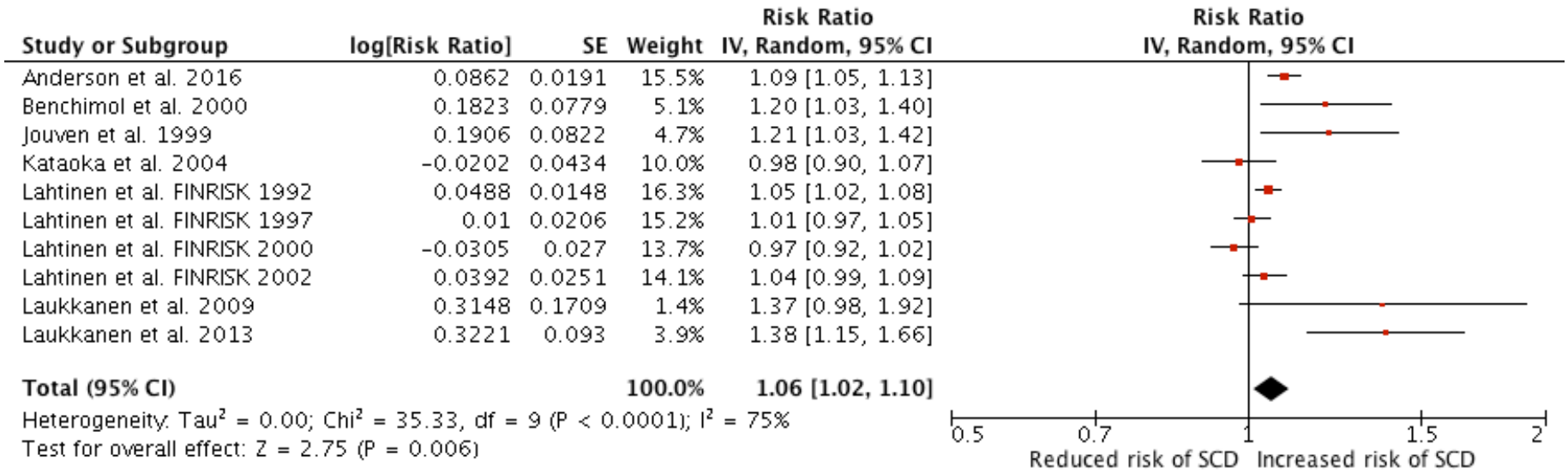


Figure 3. Risk of Sudden Death in Underweight Subjects

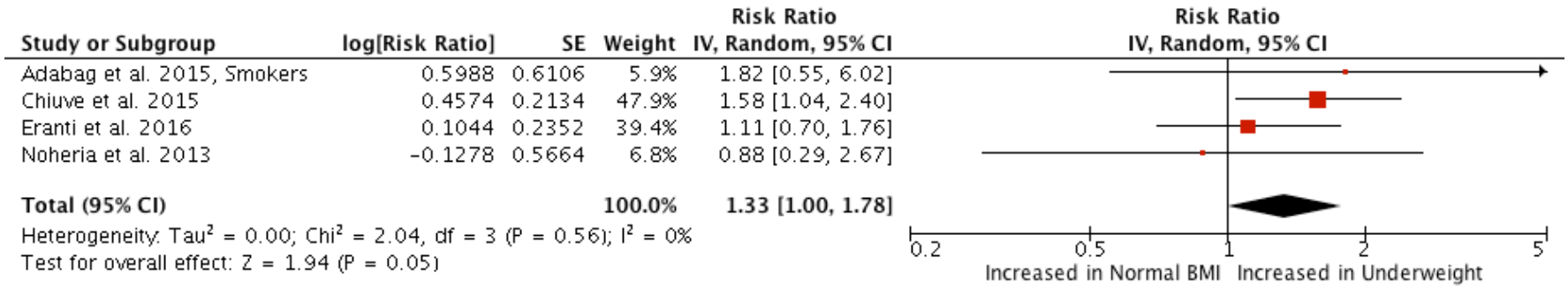


Figure 4. Risk of Sudden Death in the Overweight Subjects.

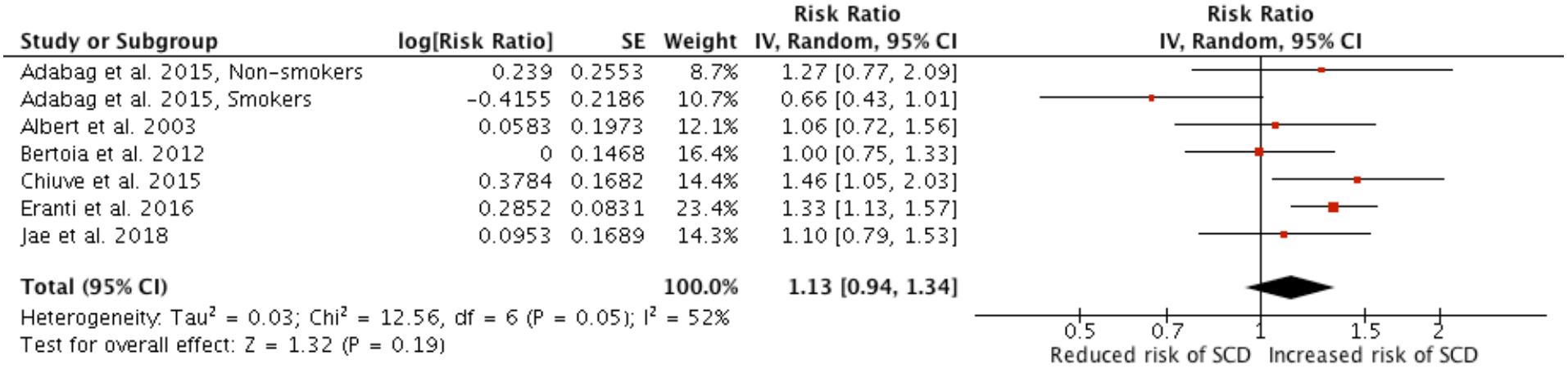
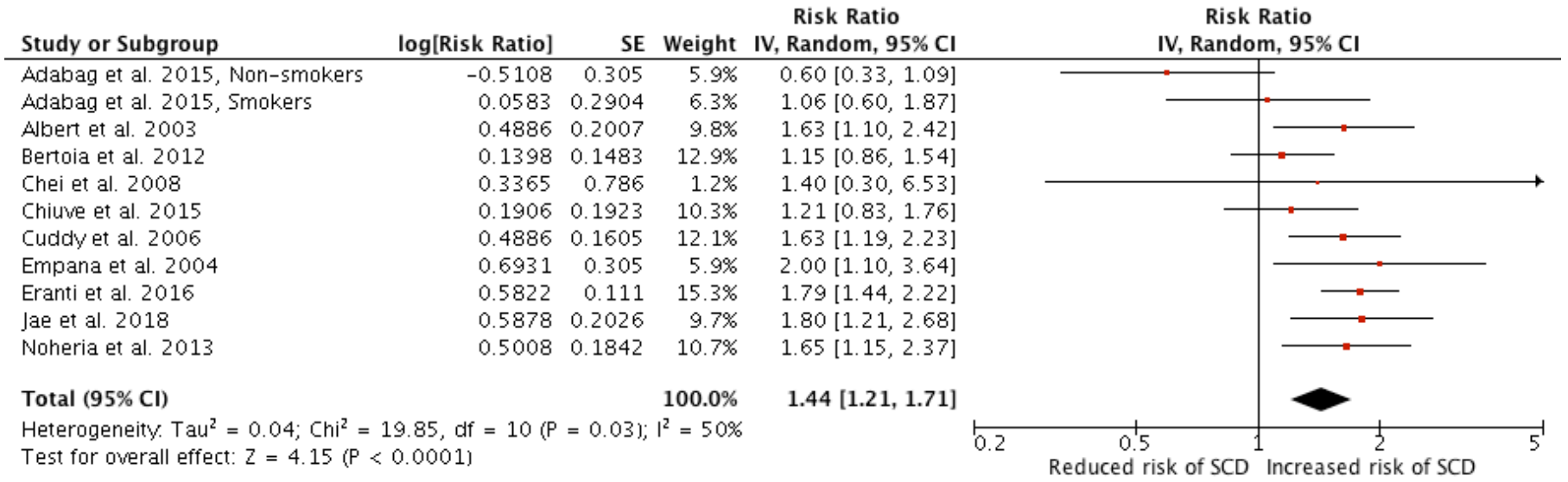
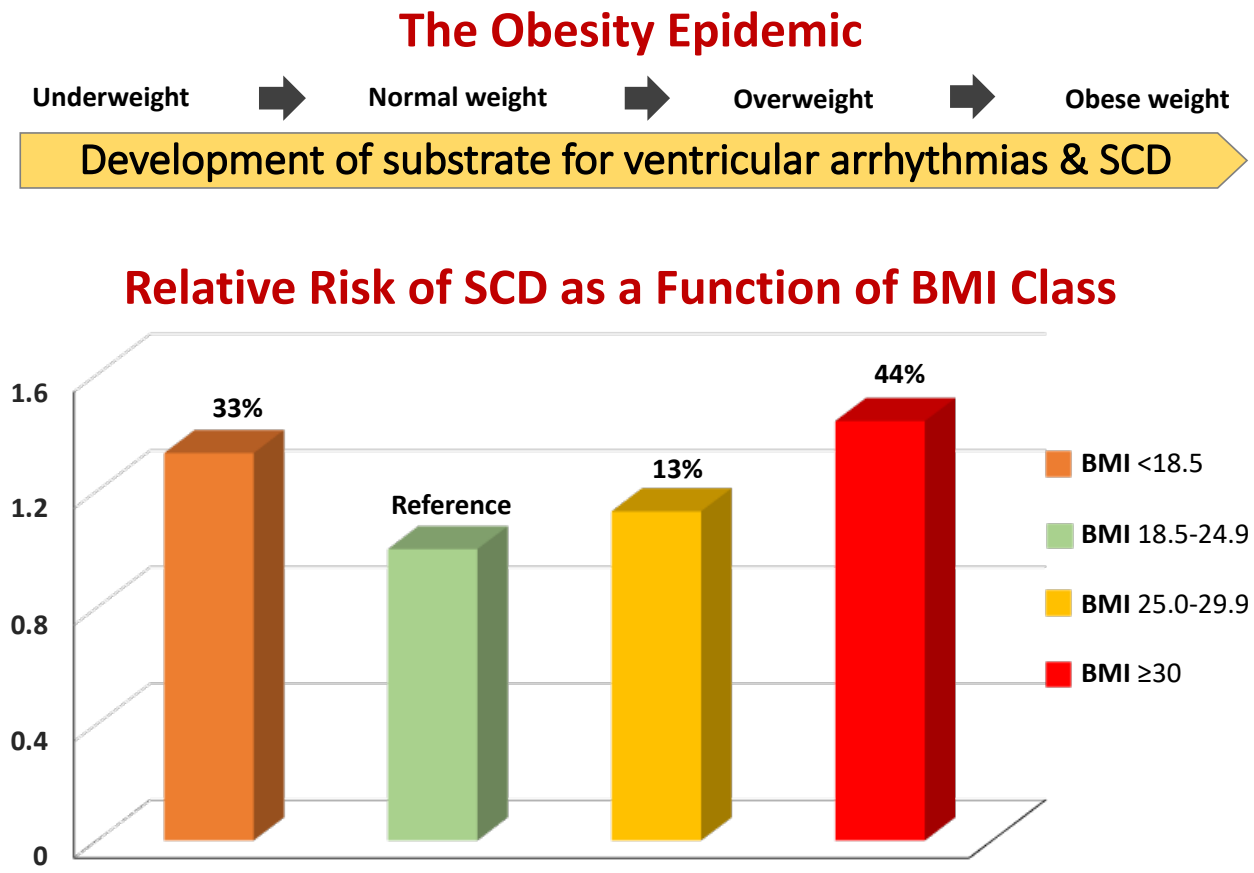


Figure 5. Risk of Sudden Death in the Obese Subjects.



Take home figure. Schematic of SCD risks in different weight subclasses



7. Chapter Seven

Epicardial Fat and Fibro-fatty Infiltration of the Ventricle: Implications for Sudden Cardiac Death Substrate in Obesity

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7.1 INTRODUCTION

Sudden cardiac death (SCD) is a major health conundrum worldwide, accounting for more than 50% of all cardiac deaths.[309, 310, 312, 425] Notably, very little has changed in the outcomes after SCD over the last few decades. Although there has been a gradual reduction in overall cardiac deaths, SCD-attributable cardiovascular death has demonstrated a steady increase.[14, 458] In the US alone, SCD has been shown to cause 180,000 to 450,000 events and is estimated at 50 to 100 per 100,000 individuals in both North America and Europe.[14, 316]

A large proportion of SCD events are attributed to ischaemic heart disease, cardiomyopathies (CMP) and inherited channelopathies.[459, 460] Indeed, SCD can be the first manifestation of ischaemic heart disease.[426] Despite the declining burden of some of these predisposing conditions, deaths due to SCD has remain unchanged or, in some areas, is rising.[427, 461-463] Moreover, in many suffering an SCD episode, there are no salient high-risk features to have prompted a preventative strategy.[331, 425]

In parallel, there is the growing global obesity epidemic, which poses a huge socio-economic challenge and a significant disease-attributable public health burden.[1] Recent clinical reports have demonstrated obesity as a strong independent predictor of greater risk for SCD.[464, 465] A body mass index (BMI) >30 $kg.m^{-2}$ has been associated with late potentials on signal-averaged ECG and an increased risk for developing ventricular arrhythmias.[449] It is likely that obesity drives the development of SCD independent of myocardial ischemia. In fact, a recent study has implicated obesity-mediated CMP as an important non-ischaemic cause of SCD.[339] However, the mechanism by which obesity predisposes to SCD remains poorly understood. We hypothesize that obesity results in structural change within the ventricle to account for the mechanistic link between obesity and SCD. The aim of the

current study was to characterise the molecular and structural ventricular remodelling that occurs due to chronic obesity.

7.2 METHODOLOGY

7.2.1 Study Animals

The study comprised sixteen 1-year old Merino Cross Wethers (*Ovis aries*) studied in accordance with guidelines outlined in the "Australian Code for the Responsible Conduct of Research, 2007 (the 2007 Code)" adopted jointly by the National Health and Medical Research Council, the Australian Research Council and Universities Australia. Study protocol and animals were approved by the animal research ethics committees of the University of Adelaide and the South Australian Health & Medical Research Institute, Adelaide, Australia, which adhere to the Guidelines for the Care and Use of Animals for Research Purposes.

7.2.2 Obesity Model

Obesity was induced in 16 sheep using a previously well characterised protocol.[181, 182] In brief, sheep were commenced on a high-calorie diet for a period of 36 weeks and maintained in this state for another 36 weeks. Obesity induction was started at baseline, whereby healthy sheep with normal weight were put on a diet consisting of energy-dense soy-bean 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. Pellets were gradually introduced at 8% excess basal energy requirements and rationed to 70% of total dry-matter

intake. Blood samples were periodically collected to ensure electrolyte and acid-base homeostasis.

7.2.3 Lean Controls

Another 8 age-matched sheep were maintained as controls by providing high-quality hay *ad libitum*. Baseline weight was maintained by ensuring a very low level of energy-dense pellets, which were rationed at 0.75% of body weight. Except for the difference in amount of food intake, the nutritional content of food and housing conditions were identical for both the obese and control groups.

7.2.4 Animal Preparation

Animals were pre-acclimatized for at least 1 week before any surgery. Shorn weight was recorded immediately before surgery. Noteworthy, we could not continue with the electrophysiology studies after initial observations of high mortality rates in the obese group (half died due to sudden deaths).

7.2.5 STUDY PROTOCOL

7.2.5.1 Structural and Functional Evaluations

The obese and the control groups underwent the following investigations:

7.2.5.1.1 Body Composition

The total body fat was quantified using the dual-energy X-ray absorptiometry (DEXA) scanning protocol under sedation.

7.2.5.1.2 Haemodynamic Assessment

Invasive blood pressure (BP) monitoring was performed during the electrophysiology study. Left atrial (LA), right atrial (RA), and pulmonary artery (PA) pressures were recorded, respectively.

7.2.5.1.3 Cardiac MRI

Before open chest surgery, animals underwent cardiac MRI using 1.5 Tesla (Siemens Sonata, MR Imaging Systems, Siemens Medical Solutions, Erlangen, Germany) with 10-mm slices through the ventricles without interslice gaps. To do this, animals were securely placed in the dorsal recumbent position for scanning. Mechanical ventilation was maintained, facilitating electrocardiogram-gated image acquisition with periodic breath holding. Analyses were performed offline by blinded operators by using the proprietary software QMass MR (Medis medical imaging systems, Leiden, The Netherlands). The following parameters were measured as previously described: Left ventricular chamber mass; LV ejection fraction (LVEF); LV end diastolic volume (LVEDV); and epicardial fat volumes.

Quantification of EAT: Epicardial fat volumes were quantified using previously validated protocol.[413] Briefly, a 3D model was constructed from consecutive end-diastolic short-axis images using semi-automated software. Regions of adipose tissue were marked in each slice followed by linear interpolation of pixel intensities in spaces between consecutive image

slices. Periatrial and periventricular fat were defined as any pericardial fat subtending the atria and ventricles and below the visceral pericardium, respectively. Total volume of adipose tissue was calculated as a total volume of the 3D model and the mass estimated from volume measurements. The Intra-observer and inter-observer reproducibility demonstrated a coefficient of variation 8.3% to 10.7% for atrial EAT, and 6.6% to 7.4% for ventricular EAT, and 5.5% to 7.2% for total EAT, respectively.

7.2.5.1.4 Transthoracic Echocardiography

Echocardiogram was performed with Acuson Aspen (Siemens Healthcare, Malvern, Pennsylvania) under general anaesthesia. The LV dimensions were determined in the M-mode in the parasternal long-axis view at the level of the mitral leaflet tips. Using the Teicholz formula, we measured global LV function from the LV dimensions.

7.2.5.2 Morphological Evaluations

Following the imaging studies, the sheep were maintained under general anaesthetic. Thereafter, they were then euthanised by lethal dose of phentobarbitone injection and removal of the heart, with samples taken for detailed histological and ultrastructural analyses. Confirmation of death was done via visual inspection of a lack of heartbeat.

7.2.5.2.1 Histomorphometric Assessment

The animals were euthanised and the hearts tissue was preserved in 10% (w/w) formaldehyde. Tissue sections were taken from the right and left ventricular free walls and

embedded in paraffin wax. Fixed tissue blocks were cut in 5-µm serial sections and stained with haematoxylin and eosin (H & E) and Masson Trichrome stains, respectively.

7.2.5.2.1.1 Assessment of Ventricular Fatty Infiltration

The fat cell infiltration of the ventricles by the epicardial fat was confirmed in Oil O Red preparations and assessed in H & E stained sections. Slides were scanned by NanoZoomer digital image scanner and viewed on NDP.view 2 (Hamamatsu Photonics K.K., Japan). Evaluation of infiltration was done at low-power (1.2x) magnification and with grading algorithm developed differently for the LV and RV.

For the LV, infiltrated adipocytes were graded as a function of the distance away in millimetre away from the epicardial surface of the myocardium.

- Grade I: For none or less than 1 mm extension from the epicardial surface of the muscle wall.
- Grade II: For infiltration extending beyond 1 mm but less than 2 mm.
- Grade III: For infiltration of more than 2 mm but less than 3 mm.
- Grade IV: For infiltration of 4 mm or more of the wall of the LV.

For the RV, grading was done by the anatomical landmark of the muscle wall from 1 to 4 as follows:

- Grade I: For no none or focal infiltration of the adjacent outer third of the ventricular wall by epicardial adipocytes.
- Grade II: Coalescent infiltration of the outer third and/or focal infiltration up to the middle third of the ventricular layer.
- Grade III: Coalescent infiltration extending from the epicardial adipose tissue to the middle or inner third of the ventricular muscle layer.

- Grade IV: For up to the sub-endocardial surface.

7.2.5.2.1.2 Assessment of Ventricular Fibrosis

5-µm sections of ventricular tissue were stained with Masson's trichrome and scanned by Hamamatsu NanoZoomer digital image scanner. Images were acquired at high-power (6.4x magnification) and analysed using purpose-built macros by colour deconvolution in Image *J* software. 20 images taken per sample were analysed from 8 animals per group to determine the mean values.

7.2.5.2.2 Immunohistochemistry

To assess fibrotic pathways, immunohistochemistry was performed. Isolated LV and RV tissues were fixed in 10% neutral formaldehyde, embedded in paraffin and cut in 5-µm sections. Embedded sections were deparaffinised by heat and subsequent washes in silane and absolute ethanol. Endogenous peroxidases were masked by incubation in 0.3% hydrogen peroxidase in methanol for 30 minutes. After performing heat-induced epitope retrieval, sections were incubated in normal horse serum for non-specific sites block, and incubated overnight with the appropriate primary antibodies against:

1. Anti-angiotensin II receptor subtype 1 (AT_1R, Rabbit polyclonal, 1/800 dilution, Biorbyt Ltd);
2. Mineralocorticoid receptor (MCR, mouse monoclonal, 1/800, Abcam);
3. Endothelin receptor type A (ET-A, Rabbit polyclonal, 1/400, Sapphire Bioscience Pty);

4. Transforming growth factor beta type 1 receptor (TGF-βR1, Rabbit polyclonal, 1/400, Sapphire Bioscience Pty);
5. Phosphorylated *sma and mothers against decapentaplegic* homologue protein 3 (pSMAD3, Rabbit polyclonal, 1/500, Biorbyt);
6. SMAD6 (Rabbit polyclonal, 1/1000, ThermoFisher Scientific); and
7. Desmosomal disruption was assessed by evaluation of desmoglein-2 expression (DSG2, Rabbit polyclonal, 1/250, Biorbyt Ltd) to understand mechanism of fatty infiltration.

Next, sections were incubated in appropriate biotinylated secondary antibodies (Goat anti-rabbit/mouse, Abcam) for 30 min at 1/250 dilution, followed by 1-hour incubation in streptavidin horseradish peroxidase-conjugated tertiary antibody at 1/1000 dilution. Immunoreactivity was evaluated using 3,3'-diaminobenzedine (DAB, Sigma) for 7 min and counter stained with Mayer's Haematoxylin. Sections were scanned using NDP NanZoomer digital scanner and viewed on NDP.view 2. Images were captured and exported at 20x magnification (100 μm, 100% scale), and semi-quantitatively assessed by colour deconvolution in Image *J* software.

7.2.6 Statistical Analysis

Normally distributed continuous variables were presented as mean ± SD and analysed using 2-tailed independent student *t*-test. Skewed data (such as endothelin A and SMAD6 protein expressions) were expressed as median and interquartile ranges and analysed using Mann-Whitney U tests. Nominal variables (such as infiltration grades) were assessed using Pearson X^2 tests. Next, we fitted mixed-effect models to the data to compare pro-fibrotic markers and

desmoglein-2 across chambers and groups (control and obese). Animal group (control and obese) and chamber (RV & LV) were modelled as fixed effects with an interaction term (chamber x group). If a significant interaction was present, mixed-effects post-hoc test p-values were reported (with Sidak adjustment of alpha level). In the case of skewed distribution data were log-transformed before further analysis. Statistical significance was defined at 2-sided p-value ≤0.05. All data analyses were performed in SPSS software package version 25 (IBM SPSS Statistics, Chicago, Illinois, USA) and GraphPad Prism version 7.0d (GraphPad Software, La Jolla, CA, USA).

7.3 RESULTS

7.3.1 Animal Characteristics

The obese state was achieved over 72 weeks, with the obese group reaching peak weight (94.71±6.5 kg) by the 36th week and sustained at the achieved weight for another 36 weeks. The control group maintained lean weight (57.4±4.6 kg) over the 72-week period. By the end of 72 weeks, the obese sheep significantly increased their baseline weight to almost twice the baseline levels ($p<0.01$). There was over a 3-fold increase in total body fat composition in the obese state ([35% of body weight versus 9.9%; $p<0.001$] **Table 1**).

7.3.2 Structural and Functional Remodelling

LV septal dimension was higher in the obese group as compared to controls ($p<0.01$) without any change in LV function between the groups ($p=0.11$). There was elevation of the left atrial

(LA) mean pressure (p=0.01) without change in systolic blood pressure (p=0.5) in the obese sheep; suggestive of diastolic dysfunction.

7.3.3 Epicardial Fat Hyperplasia and Fat Cell Infiltration

With sustained obesity, epicardial fat significantly increased to more than 2.5-fold those of the lean controls as determined by CMR (p=0.04). Accordingly, we explored epicardial fatty infiltration using H & E staining under low power. As shown in **Figure 1 panel A**, there was clear demarcation between epicardial layer and the muscle layer in the controls; however, with sustained obesity, the epicardial adipocytes infiltrated deep within myocardium causing dislodgement of the muscle cells. The fatty infiltration was quantified semi-quantitatively. There was more extensive infiltration of epicardial adipocytes in the LV of the obese sheep than the control group (mean grade: 3.00±0.9 vs 1.66±0.5; p=0.03; **Figure 1 panel B**). Severe (Grade IV) infiltration was seen in the LV of the obese group, with absence of grade III infiltration in controls. Similarly, the RV demonstrated greater infiltration by fat cells in the obese animals as compared to controls (mean grade: 3.17±1.2 vs 1.83±0.9; p=0.03; **Figure 1 panel B**), with infiltration extending up to the sub-endocardial surface (obese = grade III&IV vs control ≤ grade II).

7.3.4 Desmosomal Disruption

DSG2 expression was assessed with immunohistochemistry. DSG2 expression was significantly reduced in the obese group; with 15% reduction in protein levels in both RV and LV in obese group as compared to controls (RV:46.6±3 vs 54.1±7, p=0.04; LV: 40.2±1.1 vs 47.8±4.9%, p=0.02; **Figure 2 panels A & B**). The expression of DSG-2 demonstrated

significant correlation with animal weight and degree of fatty infiltration in linear regression models ([weight: R^2=0.424, p=0.02] and [fatty infiltration: R^2=0.527, p<0.001]; **Figure 2 panel C**). Animals with the most severe infiltration demonstrated the most significant reduction in DSG2.

7.3.5 Ventricular Fibrosis

The obese sheep demonstrated induction of diffuse interstitial fibrosis in both chambers (**Figure 3 panel A**). Analysis of the Masson's trichrome stained sections of the LV showed significantly greater percentage fibrosis as compared to the lean sheep (13.2±2.8% versus 5.2±0.9%, p=0.01; **Figure 3 panel B**). Comparable findings were seen in the RV myocardium, with obese sheep demonstrating a significant 14.8±6.1% global fibrosis as compared to 5.9±0.9% induced fibrosis in the control animals (p<0.01).

7.3.6 REMODELLING OF FIBROTIC PATHWAYS

7.3.6.1 Transforming Growth Factor-Beta (TGF-β) Pathway

Figure 4 demonstrates the summary data on the assessment of TGF-β pathway, with the model signalling pathway shown in **panel A**. There was an abundant expression of the receptor protein of TGF-β1 in both ventricles as shown in panel B. TGF-β type 1 receptor (TβR1) expression was significantly increased in the LV of obese sheep, with up to 3-fold change as compared to the control lean weight sheep (13.4±7.2% versus 5.6±1.6, p=0.02; **Figure 4 panel C**). Similarly, the expression of TβR1 was upregulated in the RV in the obese group as compared to controls (16.9±5.6 vs 4.7±2.0%; p<0.01).

Further, we evaluated the downstream signalling of TGF-β. Contextually, TβR1 transmit pro-fibrotic signals downstream by phosphorylating SMAD (*Sma and mothers against decapentaplegic homologue*) proteins, notably, SMAD3, which ultimately regulates pro-fibrotic genes and fibrosis (**Figure 4 panel A**). As can be seen **Figure 4 panel C**, there was no significant alteration of phosphorylated SMAD3 with sustained obesity ([p>0.05 for both RV & LV], **Figure 4 panel D**), indicating that this component of the pathway may not drive obesity-induced TGF-β signalling. On the other hand, there was significant remodelling of SMAD6, the inhibitor of the SMAD3-independent component of the pathway (Mann Whitney p=0.001]; **Figure 4 panel E**). The control sheep demonstrated abundant expression of SMAD6 protein, which exhibited nuclear localisation. Intriguingly, chronic obesity resulted in a substantial repression of SMAD6 expression and nuclear localisation in both the LV and RV ([RV: 2.6±0.4% versus 6.9±0.9%, p<0.01] and [LV: 1.8±0.3% versus 5.8±1.3%, p<0.01]; respectively), **Figure 4 panel E**.

7.3.6.2 Endothelin 1 Signalling

Obesity was associated with overexpression of the cognate receptor for endothelin signalling (ET-A) in both ventricles (**Figure 5 panels A & B**). As compared to the lean controls, chronic obesity resulted in increased upregulation of ET-A protein to nearly twice the baseline levels in both the LV (47.6±6 versus 26.8±6.2; p<0.01) and the RV (52.7±2.5 versus 30.3±5.8; p<0.01; **Figure 5 panel C**).

7.3.6.3 Aldosterone Signalling

Mineralocorticoid receptor (MCR) for aldosterone changed appreciably with chronic weight gain (**Figure 5 panels A & C**). This changed from 43.4±6.2% in the lean controls to 55.0±3.3% in the LV of obese group ($p<0.01$). Similarly, MCR protein was upregulated in the wall of RV (54.4±3.1 versus 41.1±7.6; $p<0.01$).

7.3.6.4 Angiotensin II Signalling

The pro-fibrotic type 1 receptor for Ang II (AT_1R) was found to be markedly expressed with obesity (**Figure 5 panels A & D**). Obesity resulted in more than 4-fold elevation of AT_1R protein levels in the LV compared to lean controls (12.1±4.9 versus 3.7±0.6; $p<0.01$). In the LV, significant expression of AT_1R was also observed, chronic obesity associated with doubling expression levels compared to maintained baseline weight (10±3.4 versus 4.2±1.8; $p<0.01$).

7.4 DISCUSSION

7.4.1 Major Findings

The present study provides new mechanistic insights into the nature of ventricular substrate for SCD in obesity. Using a chronic ovine sheep model, ventricular remodelling due to chronic obesity was characterized by:

1. Expansion ventricular epicardial fat depot (2.5 fold);
2. Extensive and severe fat cell infiltrations;
3. Diffuse ventricular interstitial fibrosis;

4. Reduction of the expression of ventricular desmosomal cadherin desmoglein-2 (15% reduction), with significant negative correlation with degree of fatty infiltration;
5. Modulation of the TGF-β pathway, with greater expression of TGF-β receptor protein (2-4 fold); downregulation of the anti-fibrotic SMAD6; and no significant alteration in pSMAD3 levels;
6. Overexpression of Angiotensin II receptor subtype 1; Aldosterone receptor protein, MCR; and endothelin receptor protein, ET-A.

These important structural consequences to the ventricular myocardium may in part contribute to the development of SCD in obese individuals.

7.4.2 Obesity and SCD

Obesity has been shown to predict greater risk for SCD. Both overweight and obese BMI's are reported to contribute from 33% to 79% elevated risk of premature death due to SCD.[436] Sub-analysis of the MADIT II trials showed significant association between obesity and ventricular tachycardia/fibrillation, and was shown to persist even after correcting for other clinical comorbidities.[428] Moreover, BMI >25 $kg.m^{-2}$ is positively correlated with higher incidence of non-sustained VT and 33% higher risk of exercise-induced VTAs independently of covariates.[428, 466, 467] SCD requires an electrical trigger acting on vulnerable substrates to generate lethal ventricular tachyarrhythmias.[309, 322, 458] QRS fragmentation (fQRS), which represents subtle scarring and myocardial substrate, has been described to be more common in obese SCD victims as compared to normal weight groups.[454] Similarly, obesity has been

correlated with late potentials, signifying delayed activation in diseased myocardium and known marker for SCD, on signal-averaged ECGs.[429]

7.4.3 SCD Substrate in Obesity

The substrate for ventricular arrhythmias in obesity is not well investigated. In the summary **Figure 7**, a postulate based on the current study and available evidence is provided to suggest the mechanisms for the substrate for SCD in obesity.

Obesity is associated with ventricular remodelling consisting of increased LV diameter and mass, eccentric hypertrophy, diastolic dysfunction, and repolarisation abnormalities.[457] Additionally, LV systolic dysfunction[468] and RV diastolic dysfunction[457] have been reported in the severely obese individuals. Furthermore, weight loss has been shown to be associated with improvements in diastolic function and LV mass, and RV systolic function.[451] Our data was in line with previous reports[457] and confirmed that severe sustained obesity is associated with elevation of left atrial pressure in the absence of systemic hypertension.

Fibrosis plays an important role in the development of arrhythmias and is regarded as the histological cornerstone of structural remodelling that creates a substrate for arrhythmias.[327, 455] Similarly, recent reports suggest significant association of epicardial fat expansion with increased frequency of premature ventricular contractions[469], VT/VF[470] and all-cause long-term mortality[471] and mortality due to SCD[471]. The present study demonstrates increase in interstitial fibrosis with severe obesity, similar to non-ischemic cardiomyopathies.[455] Furthermore, we also demonstrate the novel finding of fatty infiltration in the ventricles with severe obesity. We hypothesize, that akin to fibrosis, fatty infiltration

may predispose to conduction heterogeneity, re-entry and ventricular arrhythmias. Consistent with this, Pouliopoulos et al[472] reported fibro-fatty interfaces within LV scar borders is significantly associated with altered electrophysiological remodelling and abnormal Cx43 expression and that gradient increase in intramyocardial adiposity correlates with increased inducibility of VT following MI.

Desmosomal mutations have been shown to result in fibro-fatty infiltration of the ventricles in arrhythmogenic right ventricular dysplasia (ARVD) by compromising myocyte adhesion and loss of further desmosomal proteins.[473] In the present study, we observed a similar, but to a lesser extent, reduction in the expression of desmoglein-2 (DSG2), a desmosomal cadherin whose mutation is found in up a third of ARVD patients[474]. We propose that this reduction in DSG2 expression is the mechanistic link responsible for infiltration of ventricular muscle by overlying epicardial adipose tissue. Previous studies have shown that the epicardial adipose tissue secretes pro-fibrotic adipokines.[278] The fatty infiltration could potentially potentiate the paracrine role of the epicardial fat by increasing the exposure of the cardiomyocytes to the pro-fibrotic factors.

7.4.4 Molecular Mechanism of Fibro-fatty Infiltration

The current study demonstrated activation of TGF-β, endothelin and RAAS pro-fibrotic pathways in severe obesity. TGF-β signalling is considered a central pathway in fibrogenesis.[125, 475] The present study demonstrates that activation of the TGF-β pathway with associated with downregulation of SMAD6, but not pSMAD3 protein. Based on these findings, we propose that obesity-mediated TGF-β signalling is likely to be via SMAD-independent component of this pathway. The current study also demonstrated activation of

the angiotensin II and aldosterone pathways by the overexpression of the pro-fibrotic AT_1R and MCR. Both Ang II and aldosterone are important effectors of RAAS system and are shown to induce interstitial fibrosis and predispose to ventricular tachyarrhythmic events.[475-477] Previous studies have shown that aldosterone can also act independent of this system, with the activation of cardiac MCR resulting in hypertrophy, fibrosis, and heart failure independent of blood pressure levels.[478] It is important to highlight the prime role of MCR, insofar as emerging data from human and *in vivo* animal studies report induction of cardiac fibrosis by the aldosterone/MCR pathway without concomitant increase in MCR ligand levels but only in receptor expression.[475, 478] Furthermore, there was overexpression of endothelin receptor subtype A (ET-A), the receptor for the vasoconstricting endothelin 1 (ET-1). ET-1/ET-A acts a downstream target of TGF-β gene transactivation and angiotensin II-induced fibrosis, and as an amplifier of the pro-fibrotic cascade.[479]

7.4.5 Limitations

We present strong evidence implicating obesity in ventricular remodelling. However, few limitations should be noted. First, our data is limited by its experimental nature. Although they have important implication for the understanding of potential mechanism driving SCD in obesity, we would need more translational studies to determine the clinical applications. Second, there are several methods for assessing molecular remodelling. Here, we were only able to evaluate some key receptors and downstream effector proteins due to the availability of appropriate reagents. Like downstream effector proteins, receptors are very good indicators of local perturbations. They are also better indicators of more chronic changes and so will be more useful in assessing effects of chronic obesity. Finally, we were not able to

ascertain the electrophysiological consequences of our structural findings. This was largely to do with high incidence of sudden cardiac death experienced during the electrophysiological study model. About eight of the obese sheep (≥110 kg) experienced several episodes of ventricular arrhythmia and subsequently sudden death with handling of the obese heart.

7.4.6 Clinical Implication

These findings highlight potential mechanisms that may explain the clinical associations reported between and obesity and expansion of epicardial fat and SCD. The novel finding of fibro-fatty substrates could form a key element in substrate mapping as a guide for ablation of lethal ventricular arrhythmias. EAT may represent an interesting risk marker to identify patients with increased SCD risk which could allow a more personalized risk stratification. Further studies are warranted to improve our understanding of EAT-mediated ventricular remodelling and to determine whether its reduction constitutes a treatment target for primary and secondary prevention of SCD.

7.5 CONCLUSIONS

Chronic sustained obesity promotes biventricular remodelling driven by diastolic dysfunction, expansion of ventricular epicardial fat depot with resultant fat cell infiltrations of the ventricular wall, and diffuse interstitial fibrosis. Molecular assessments suggest that these changes due to obesity may be mediated through DSG2 and abnormal SMAD3-independent TGF-β signalling, Endothelin and RAAS activation respectively. The fibrofatty changes in the ventricles may represent a unique substrate for ventricular arrhythmias and sudden cardiac death in severe obesity.

7.6 TABLES

TABLE 1. Structural and Functional Characteristics

	Parameters	**Controls**	**Obese**	***p*-value**
	Body weight, kg	57.4±4.6	94.71±6.5	<0.001
DEXA	Total body fat, %	9.9±2.5	35.1±5.2	<0.001
CMR	Total EAT, ml	128±15	296±48	0.004
	Ventricular EAT, ml	115.5±12	253.0±53	0.04
	LVEF, %	67.6±4.5	73.2±5.2	0.11
	LV mass, g	121.0±21	138.3±18	0.39
	RVEF, %	52.7±7.5	57.5±5.9	0.422
TTE	LVEF, %	69±3.7	72±4.8	0.31
	LVSD, mm	6.8±0.4	8.0±0.6	0.003
	LVESD, mm	22.8±3.7	22.6±3.0	0.91
	LVEDD, mm	37±4.9	42.8±4.0	0.07
Haemodynamics	Systolic BP, mm Hg	69.6±12.5	74.5±12.5	0.50
	LA pressure, mm Hg	9.6±2.5	13.3±2.5	0.01

Table 2. Myocardial Deformation and Strain

	Parameters	Controls	Obese	*p*-value
LV	Longitudinal strain mean (%)	7.4±1.6	8.7±2.1	0.437
	Radial strain mean (%)	15.0±4.4	17.6±2.9	0.593
	Circumferential strain mean (%)	12.1±2.5	12.2±2.4	0.994
RV	Longitudinal strain mean, %	17.6±3.2	15.6±3.1	0.639
	Radial strain mean, %	37.9±9.4	28.1±6.6	0.138

TABLE 2. Summary of Major Pathomolecular Findings

Parameters	Left ventricle			Right ventricle		
	Control	**Obese**	***p*-value**	**Control**	**Obese**	***p*-value**
Fatty infiltration	1.7±0.5	3±0.9	0.03	1.8±0.9	3.2±1.2	0.03
Desmoglein-2 (DSG-2, %)	47.7±4.9	40.2±1.1	0.02	54.1±7	46.6±3	0.04
Percent fibrosis, %	5.2±0.9	13.2±2.8	0.002	5.9±0.9	14.8±6.1	0.01
TGF-β receptor 1 (Tβ1R, %)	5.6±1.6	13.4±7.2	0.02	4.7±2	16.9±5.6	0.001
SMAD3, %	13.5±5.9	21±4.2	0.09	19.9±12	25.2±6.4	0.45
SMAD6, %	5.8±1.3	1.8±0.3	0.001	6.9±0.9	2.6±0.4	0.001
Endothelin receptor A (ET-A, %)	26.8±6.2	47.6±6	0.001	30.3±5.8	52.7±2.5	0.001
Mineralocorticoid receptor (MCR, %)	43.4±6.2	55±3.3	0.002	41.1±7.7	54.4±3.1	0.003
Ang II receptor type 1 (AT_1R, %)	3.7±0.6	12.1±4.9	0.01	4.2±1.8	10±3.4	0.003

7.7 FIGURE LEGEND

Figure 1. Epicardial Fat Volume by Cardiac MRI

Panel A: Representative CMR images in short-axis view, demonstrating the distribution of epicardial adipose tissue in the three study groups; ventricular EAT depots are highlighted with contours. **Panel B:** Three dimensional (3D) representations of the ventricular chambers reconstructed on CMR images. **Panel C:** Bar charts demonstrating quantified volumes of EAT; showing total cardiac, total ventricular, left and right ventricular EAT volumes, respectively.

Figure 2. Fat Cell Infiltration

Panel A: Representative H&E stained sections of the right ventricle and left ventricle; and **Panel B**: degree of fat cell infiltration of obese and control sheep, respectively.

Abbreviation: LV, left ventricle; RV, right ventricle. **Data:** mean±SD.

Figure 3. Desmosomal Remodelling

Panels A, B: Relative protein expression of the desmosomal cadherin protein, desmoglein-2 (DSG2) by immunostaining, showing photo-images and semi-quantitative assessment of protein expression; and **Panel C**: showing linear regression of grade infiltration and DSG2 expression in obese animals, with data pooled from both ventricles. **Bar:** 100 μm. **Data:** mean±SD.

Figure 4. Ventricular Fibrosis

Panel A: Representative Masson's trichrome stained sections demonstrating collagen depositions of the RV and LV. **Panel B**: Quantitative data showing percent fibrosis in the myocardial tissue. **Data:** mean±SD.

Figure 5. Remodelling of Transforming Growth Factor Pathway

Relative protein expression of members of the TGF-β family by immunostaining, showing model signalling of the pathway (**Panel A**); presentative photo-image (**Panel B)** and semi-quantitative assessment of protein expression TGF-β1 receptor (**Panel C**), phosphorylated SMAD3 (**Panel D**), and SMAD6 protein (**Panel E**); Bar: 50 μm. **Data:** mean±SD. **Abbreviation**: pSMAD3, phosphorylated *sma* and mother against decapentaplegic homologue protein 3; SMAD6, SMAD protein 6; TGF-β1, transforming growth factor-beta 1; TβRI, TGF-β1 receptor subtype 1.

Figure 6. Remodelling of Further Pro-fibrotic Pathways

Further remodelling of pro-fibrotic pathways showing relative expression (**Panel A**) and semi-quantitative data for endothelin-1 receptor, (**Panel B,** ET-A); aldosterone receptor, (**Panel C,** MCR); and angiotensin II receptor, (**Panel D,** AT_1R). Bar: 100 μm. **Data:** mean±SD. **Abbreviations**: AT_1R, angiotensin II receptor type 1; MCR, mineralocorticoid receptor; ET-A, endothelin type A receptor

Figure 7. Central Illustration – Potential Mechanisms of Ventricular Remodelling in Obesity

Figure 1. Epicardial Fat Volume by Cardiac MRI

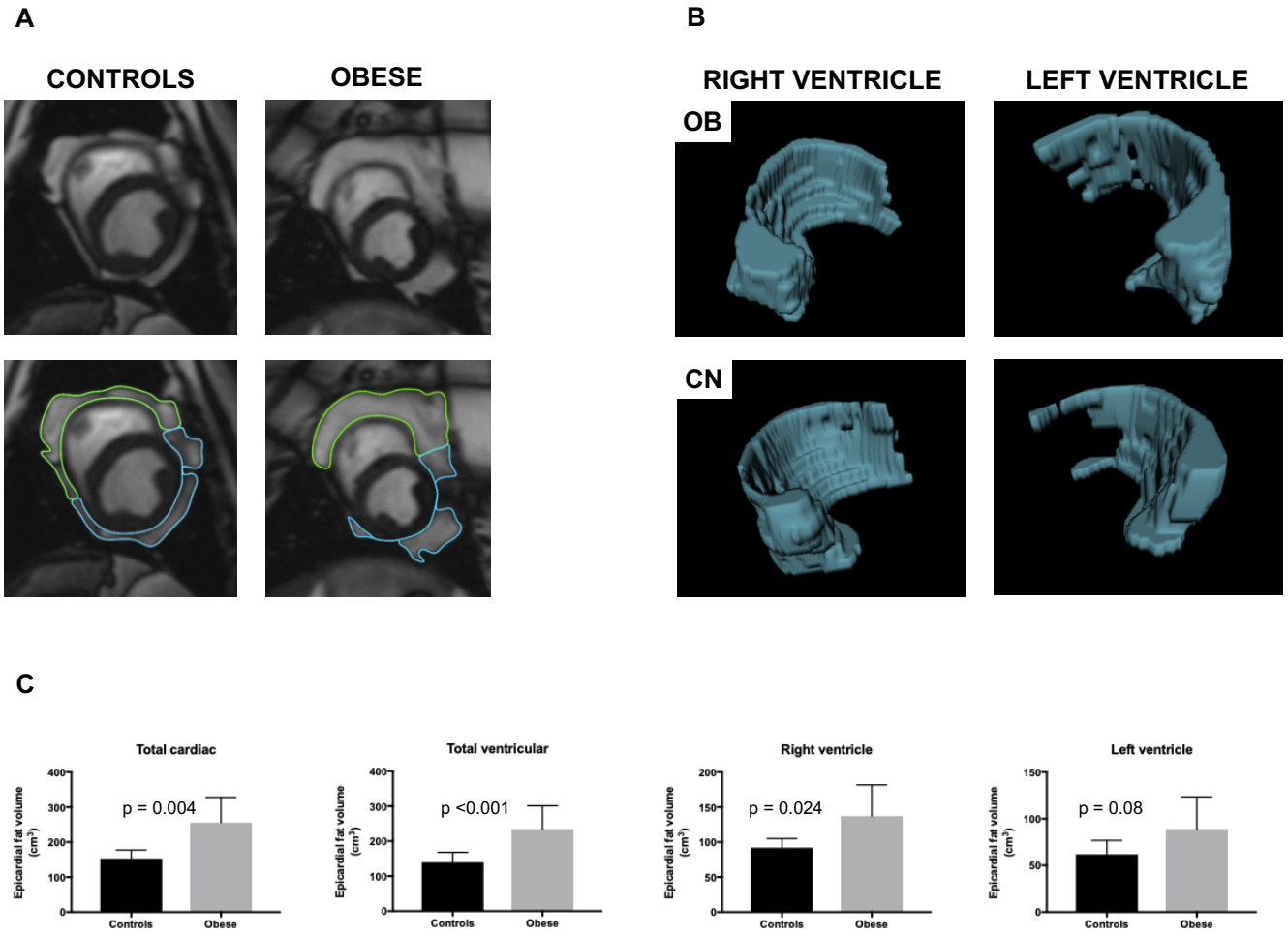


Figure 2. Fat Cell Infiltration

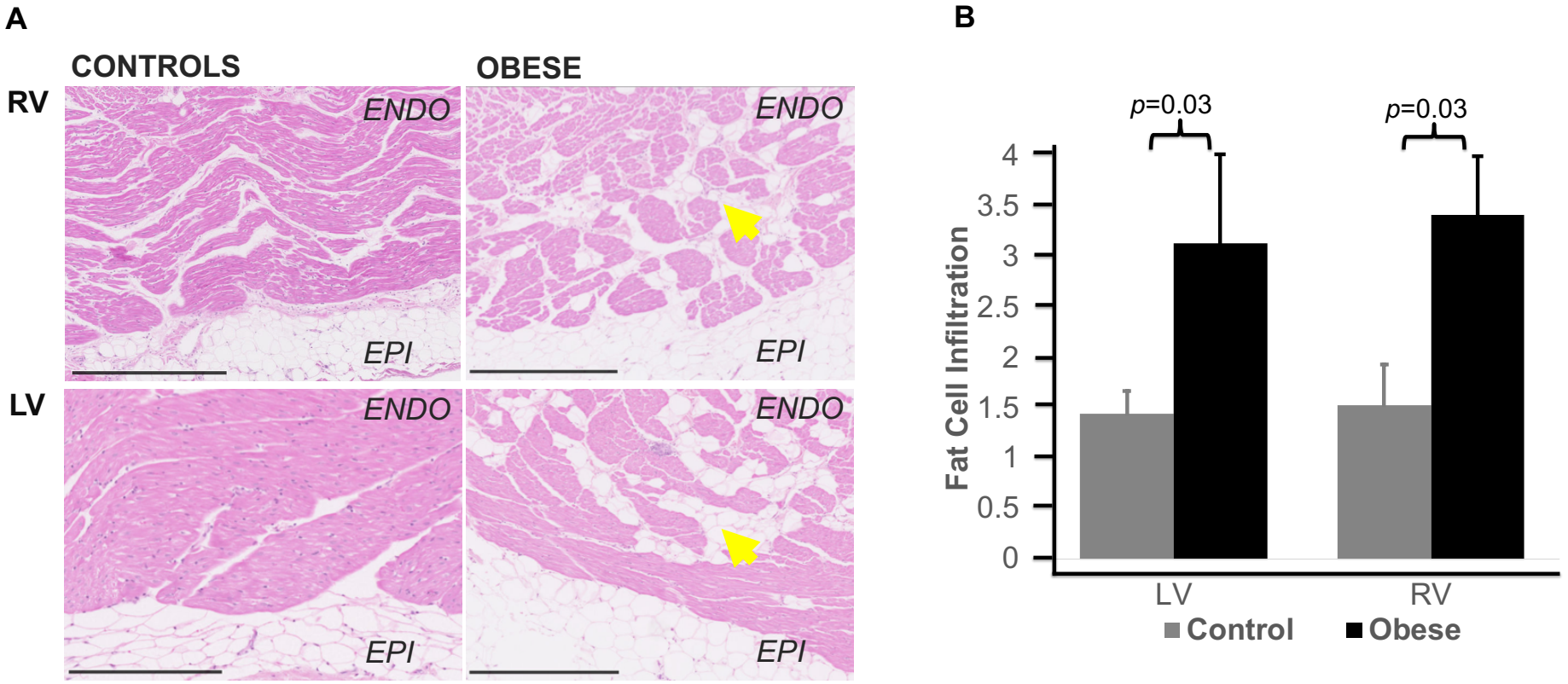


Figure 3. Desmosomal Remodelling

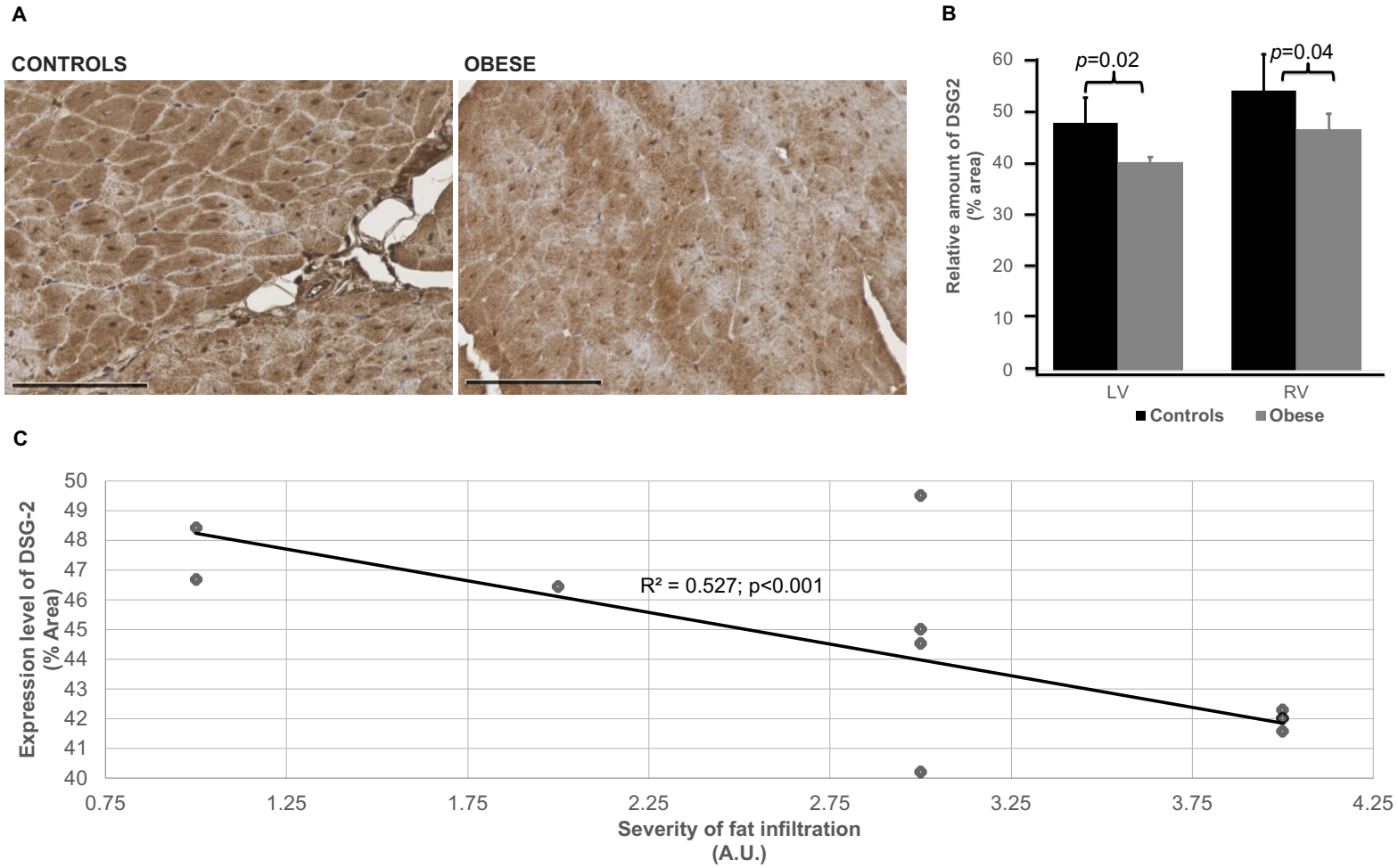


Figure 4. Ventricular Fibrosis

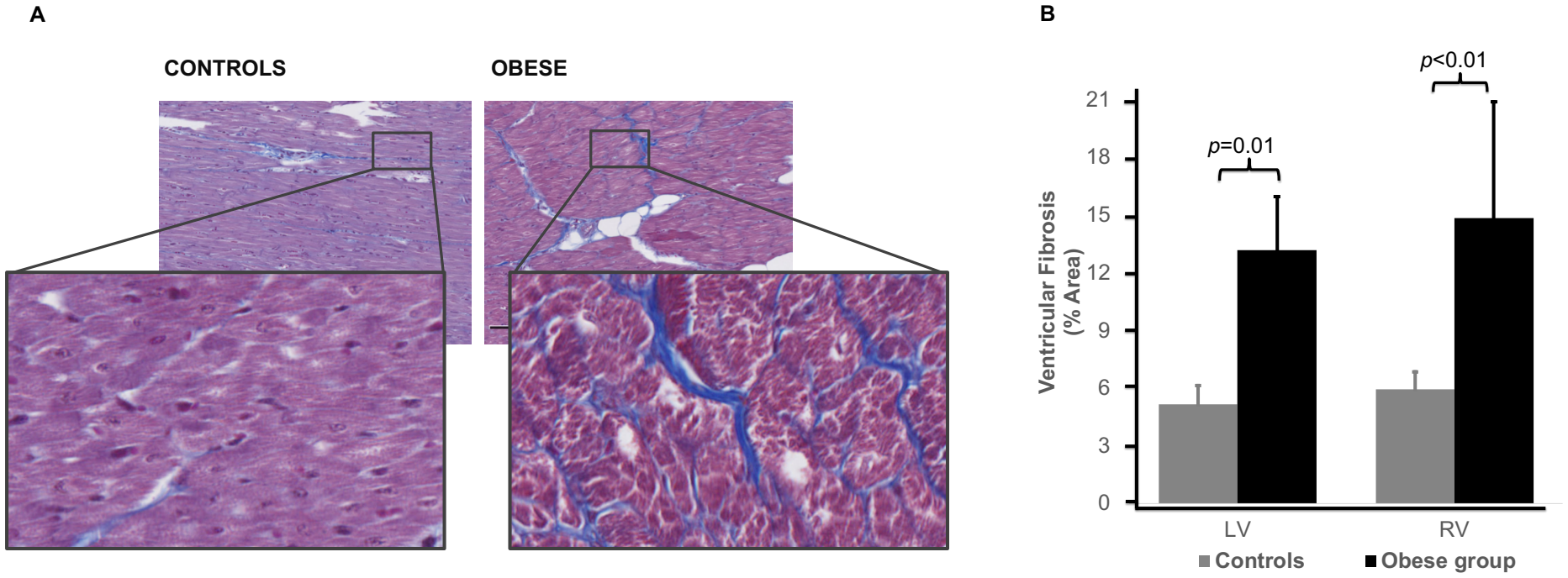


Figure 5. Remodelling of Transforming Growth Factor Pathway

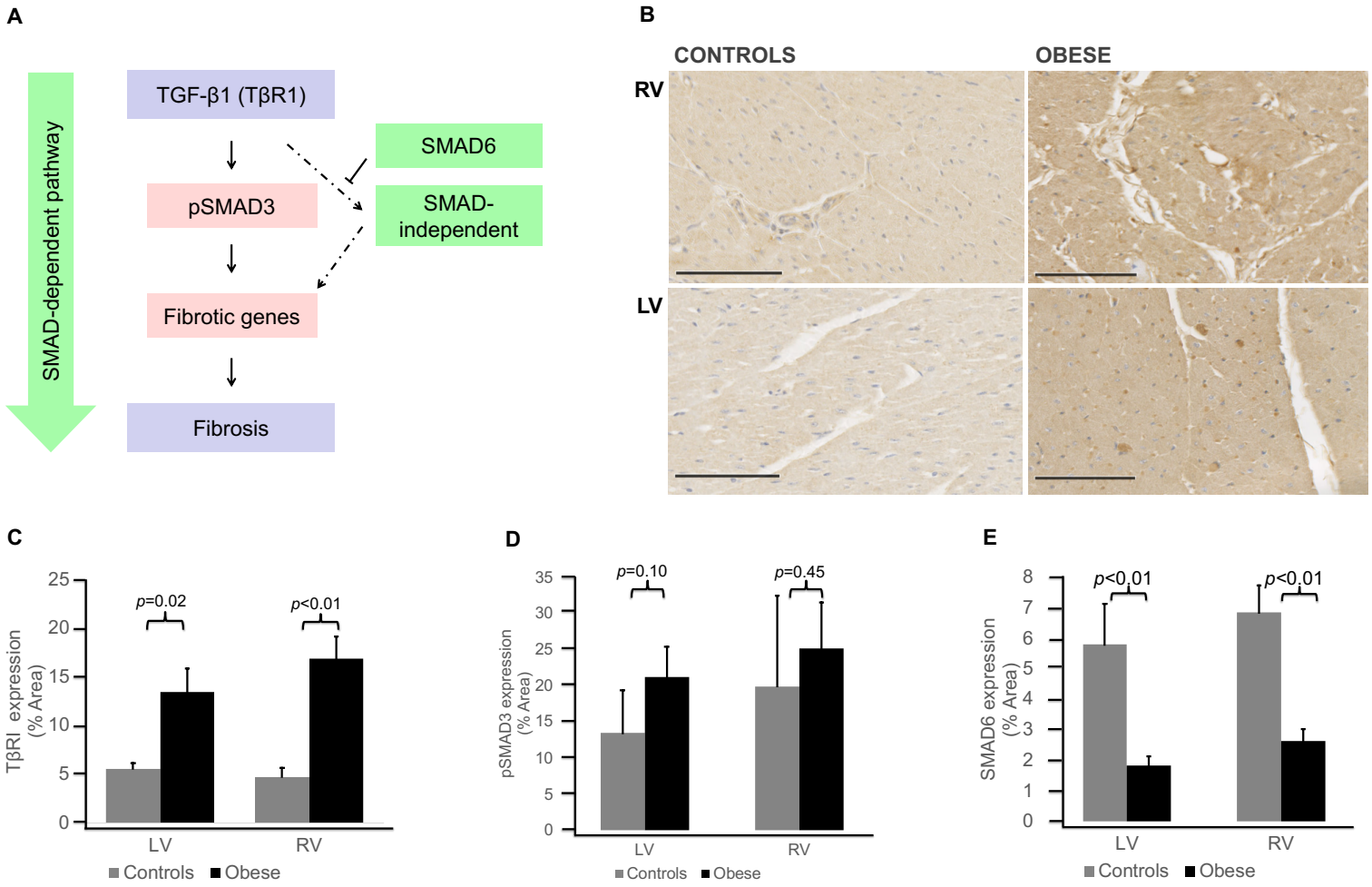


Figure 6. Remodelling of Further Pro-fibrotic Pathways

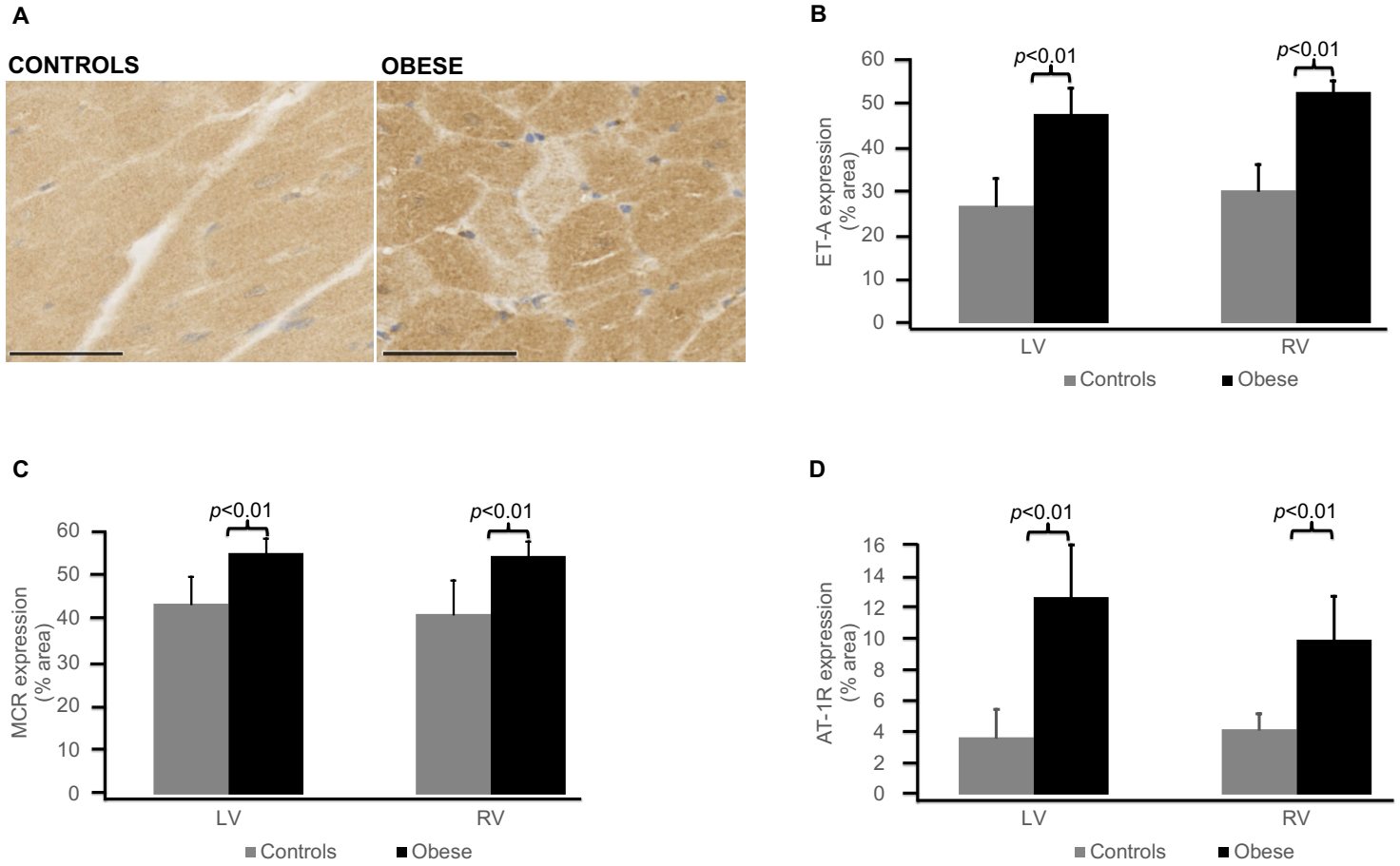
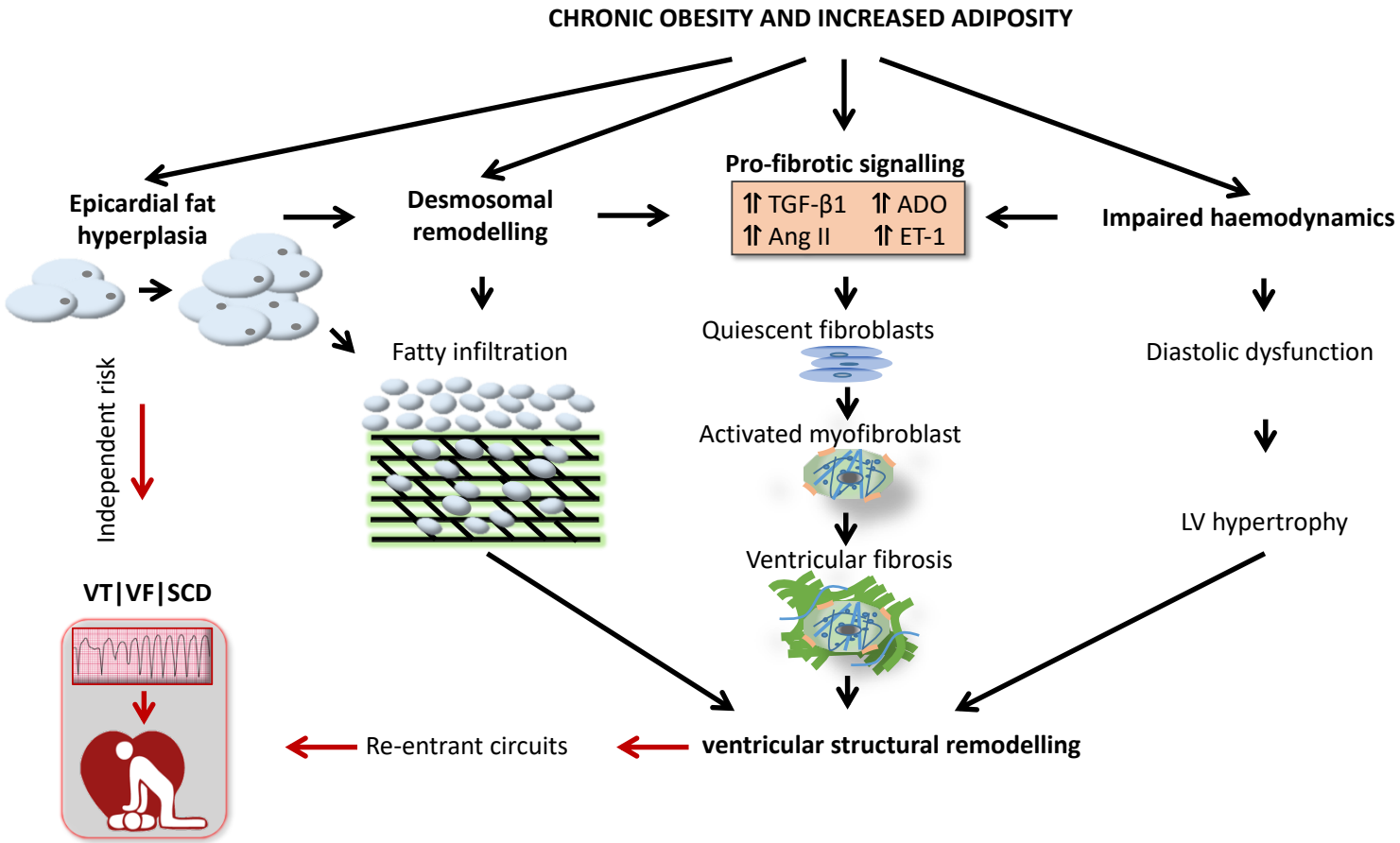


Figure 7. Central Illustration – Potential Mechanisms of Ventricular Remodelling in Obesity



8. Chapter Eight

Final Discussion and Implication

8.1 Translational Outlook

The present thesis has investigated important aspects of the developments of atrial fibrillation and sudden cardiac death in obesity, establishing epicardial fat expansion and fibro-fatty infiltrations as prerequisite events. It has provided strong clinical evidence to establish epicardial fat as an independent risk modifier for atrial fibrillation. The findings further highlight obesity as a modifiable risk marker for sudden cardiac death independent of high-risk traditional factors and conditions. More importantly, the observations provided evidence for a mechanistic link between cardiac ectopic fat and arrhythmogenic substrates, which has advanced our understanding of the mechanistic predispositions to atrial fibrillation and sudden cardiac death in obesity. They have several important implications as they underscore the need for therapeutic strategies aimed at prevention and regression of the atrial and ventricular substrates.

8.2 Epicardial Fat and Atrial Fibrillation

Multifactorial aetiological basis for atrial substrate formation is well accepted as an explanation for the atrial fibrillation epidemic. It is understood that multiple important clinical conditions (including advanced age, hypertension, heart failure, type II diabetes, valvular heart disease and obstructive sleep apnoea), electrocardiographic and echocardiographic factors, and biomarkers predispose to increased propensity for AF. Notably, data also show that AF symptomatic burden increases with increasing number of these concomitant conditions in patients, and that they associate with greater risk of chronicity of the rhythm disorder. Nevertheless, the current global burden and lifetime risk of

atrial fibrillation are more than could be explained by traditional cardiovascular risk factors alone, which highlights the need to explore emerging risk modifiers.

Obesity is implicated in precipitating AF risk and momentum is building to accurately characterise this association. Multiple lines of data have reported association of obesity with echocardiographic markers of atrial dysfunction and atrial enlargement, and markers of cardiac autonomic tone dysfunction and inflammation. Moreover, AF and obesity are parallel burgeoning global conditions, persisting even in the wake of declining traditional risk factors.

Among the factors implicated in this relation is galectin-3, a β-galactoside-binding lectin mainly secreted by macrophages and to a lesser extent by adipocytes and fibroblasts.[379] Gal-3 is has been shown to promote structural remodelling in a preclinical model of obesity. Herein, our data in Chapter 2 reports on the elegant relationship between Gal-3 and AF in a meta-analysis. We show that plasma Gal-3 is increased in patients with pre-existing AF compared to those in sinus rhythm. Our findings further demonstrated that high Gal-3 predicts greater risk of AF, this association persisting even after adjusting for covariates.

More recently, clinical reports have drawn our focus onto epicardial adipose tissue, an ectopic fat depot lying contiguously above the myocardium. It has been postulated to promote increased vulnerability to pro-arrhythmic states. Despite this, the relationship between epicardial fat and atrial fibrillation has not been properly defined. In chapter 3, the clinical associations of epicardial fat and atrial fibrillation, arrhythmia progression, recurrent atrial fibrillation following curative catheter ablation, and post-operative atrial fibrillation after cardiac surgery are presented in a meta-analysis. The findings demonstrated increased expansions of total cardiac and peri-atrial epicardial adipose, with greater risk of atrial fibrillation occurrence seen for every unit increment in epicardial fat, which persisted even after correcting for traditional cardiovascular risk factors and other measures of obesity.

Epicardial was associated with severity of atrial fibrillation, recurrence post-ablation, and *de novo* incidence after cardiac surgery. Further studies are warranted to facilitate the understanding of atrial remodelling due to EAT and determine whether its reduction constitutes a treatment target. The observations suggest possible mechanistic role for atrial fibrillation in obesity, thus warranting an understanding of the atrial substrate due to epicardial fat.

The pathogenesis of AF is driven by a complex interplay of ectopic foci and re-entrant mechanisms acting on vulnerable atrial substrates. Evidence from a number of seminal studies has identified both structural and electrical abnormalities as requisite conditions for the formation of the AF substrates. Chapter 4 & 5 draw conclusions from the investigation of the key structural remodelling underpinning epicardial fat and AF relationships and how it influences electrical substrates in chronic ovine models of obesity and weight fluctuation. It has demonstrated that obesity induces expansion of epicardial fat hyperplasia and fibro-fatty replacement of atrial myocytes and deterioration of myocyte contractile apparatus, which may drive impairments of atrial electrical properties. It has also shown that weight fluctuation induces similar but less severe changes to those seen during stable obesity and that this may explain the increased risk of atrial arrhythmias often seen with periodic fluxes in weight. Importantly, these chapters have highlighted fibro-fatty infiltration as an important substrate for the pathogenesis of atrial fibrillation, which thus necessitates therapies targeting intramyocardial fat depositions. Taken together, our data strongly support the role of epicardial fat and fibro-fatty infiltrations as important pro-arrhythmic substrates for atrial fibrillation in obesity. We speculate that, during obesity, Gal-3 may mediate epicardial fat dysfunction and downstream atrial structural and electrical remodelling. Given the deregulation of adipokines during weight fluctuation, it is possible

that Gal-3 may be involved in the formation of atrial substrate; perhaps, by acting to via leptin signalling pathways.[378, 480]

8.3 Epicardial Fat and Sudden Cardiac Death

The premature death due to sudden cardiac death represents a significant public health burden. Despite the decline in overall burden of total cardiac mortality, the proportion due to sudden cardiac has remained largely unchanged and, in some reports, has demonstrated a steady increase overtime. Moreover, population-based epidemiologic data demonstrates that half of the crude rates of SCD occur in patients without apparent high-risk features, such as myocardial infarction, inherited channelopathies, ischaemic and non-ischaemic cardiomyopathies, or impaired heart function. There is growing body of data suggesting that this burden of SCD might be driven by the rising prevalence of obesity in the community. Chapter 6 reports the findings from a systematic review and meta-analysis undertaking to define the association between obesity and sudden cardiac death. In the analysis involving over 1.4 million patients, underweight body mass index was associated with an increased risk of sudden cardiac death. Importantly, obesity predicted an exaggerated risk for sudden cardiac death even after correcting for traditional high-risk features of sudden cardiac death. Similarly, unit increment in body mass index was shown to demonstrate a greater risk for sudden cardiac death. More crucially, these findings implicate the role of obesity in the risk of sudden cardiac death and the possibility of incorporating adiposity measures in SCD risk stratification

Much akin to atrial fibrillation, sudden cardiac death is driven by abnormal cardiac electrical activities. However, the potential mechanistic drivers of the ventricular substrate for

sudden cardiac death in obesity are not known. In chapter 7, the molecular and structural substrates for ventricular arrhythmias that lead to sudden cardiac death in a model of chronic obesity are presented. Obesity demonstrated two-and-half-fold expanded ventricular epicardial fat depot with a consequent extensive and severe fat cell infiltrations. There was significant reduction in ventricular desmosomal cadherin desmoglein-2, which demonstrated significant negative correlation with the degree of fatty infiltration. Additionally, obesity was associated with induction of diffuse ventricular interstitial fibrosis. The findings further demonstrated that obesity results in significant abnormal modulation of fibrotic pathways, including an alternative component of the central transforming growth factor-beta 1 pathway, angiotensin II, endothelin and aldosterone signalling pathways.

The observation of epicardial fat expansion is particularly noteworthy as this adds an important extra layer to the stratification of patients at risk of sudden cardiac death. Indeed, there are few reports showing epicardial fat could predict greater risk for premature ventricular complexes, ventricular tachycardia/fibrillation, all-cause long-term mortality, and mortality due to sudden cardiac death in stable coronary artery disease.[469, 470] Furthermore, the novel finding of fibro-fatty substrates in the ventricular myocardium could be sufficient to create conduction blocks, leading to re-entrant ventricular tachycardia/fibrillation and sudden cardiac death.[481] More importantly, the fibro-fatty deposits could form a key element in substrate mapping as a guide for ablation of lethal ventricular arrhythmias.[282, 481]

9. Chapter Nine

Current Challenges and Future Directions

The findings made during this doctoral thesis have provided crucial understanding of the clinical and mechanistic relationships between epicardial fat and cardiac arrhythmias and sudden cardiac death. They indeed have far reaching implication for management strategies for atrial fibrillation and sudden cardiac death. However, these observations raise some important questions, which would need to be addressed in future research.

9.1 Epicardial Fat: Challenges and Concluding Remarks

The findings reported therein have demonstrated expansion of epicardial fat as a prerequisite for the development for atrial fibrillation and sudden cardiac death, using a multimodal approach involving meta-analysis and animal models. However, the incorporation of this ectopic cardiac fat in risk models is likely to be met with challenges, inasmuch as there is inconsistency in the literature regarding what constitute epicardial adipose tissue and pericardial adipose tissue. As noted in the thesis, whereas epicardial fat pertains to the fat lying contiguously with the myocardium, pericardial fat is a loose term used to describe the totality of fat depots found around the heart. It incorporates both “epicardial fat” and another adipose tissue “paracardial fat”, which is located externally to the parietal pericardial layer or membrane.[412, 413] As a drawback, ambiguity in distinguishing these fat zones could lead to overestimation or underestimation of the reported values of epicardial fat. Therefore, further studies are highly warranted to establish consensus on these definitions and clearly evaluate the risks associated with each fat depot.

Although this thesis robustly implicates mechanistic relationship between epicardial fat and sudden cardiac death, evidence for clinical association is very scant. Further studies

are warranted to thoroughly define this clinical relations and strategies to improve outcomes of patients at risk of sudden cardiac death.

9.2 Fibro-fatty Infiltrations: Opportunities for Dynamic Risk Profiling

This thesis has demonstrated extensive and severe fat cell infiltrations with induction of diffuse interstitial fibrosis because of epicardial fat hyperplasia. This represents a uniquely novel substrate for cardiac arrhythmias and sudden cardiac death due to obesity.

Use of fibro-fatty infiltrations for accurate profiling of sudden cardiac death risk in the general low-risk communities but with high events rates would make an exciting research endeavour. However, with apparent limitations in imaging modalities, clinical and non-invasive characterisation of zones of fibro-fatty infiltrates would likely be a major challenge. Nonetheless, there is some glimmer of hope, with emerging data demonstrating spatial overlap VT/VF circuits on electroanatomic maps and reconstructed CT images in the post-MI heart. Clinical utility of this approach would require extensive validation studies.

9.3 Obesity Relapse: The Bottleneck in Management Strategies

The benefits of weight loss have been demonstrated in both non-randomised and randomised trials. However, long-term sustenance of weight loss remains a major challenge in clinical practice.[344] We previously reported that 5% or more fluctuation in weight counteracts the effects of initial weight loss in reducing the burden of cardiac arrhythmias.[345] Importantly, the

findings reported in this thesis implicate an underlying remodelling in the myocardial tissue of weight fluctuation animals compared to reference controls despite having comparable amounts of epicardial fat. These observations highlight a likely induction of unique cytokine profiles by the cyclic fluxes in weight. Further research is very needed to clarify this bottleneck. More importantly, better strategies are warranted to effectively prevent relapse in obesity following weight management regimes.

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